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THE EFFECT OF AIR TEMPERATURE ON VIRUS CONCENTRATION AND LEAF MORPHOLOGY OF MOSAIC-INFECTED HORSERADISH[1]

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INTRODUCTION

Mosaic of horseradish (*Armoracia rusticana* Gaertn., Mey and Scherb.) is incited by one or more strains of turnip virus 1.[3,4] In a recent paper[4] it was shown that in plants naturally infected with mosaic, symptoms tended to become masked completely at 28° C., they were progressively more pronounced at 24° and 20°, and were severe and persistent at 16°. This was contrary to the behavior of other strains of the same virus which incited mosaic on cabbage. Symptoms caused by these were most severe at 28°, were progressively less pronounced at 24° and 20°, and tended to become masked at 16°.[5] In cabbage the concentration of strains of turnip virus 1 was correlated with the severity of symptoms, the greatest concentration occurring with the most severe symptoms.[6] It became of interest, therefore, to determine the effect of air temperature on the concentration of the horseradish virus in horseradish. In the course of the experiments it was observed that temperature had a marked effect on leaf morphology, an effect apparently independent of that on virus concentration.

MATERIALS AND METHODS

Horseradish roots were obtained from the planting stock of a commercial grower of East St. Louis, Ill. They were planted out in February 1947, in greenhouse compost in 12-inch clay pots, seven plants per pot. After 3 weeks' growth at 22° C. they were placed at constant air temperatures of 16°, 20°, 24°, and 28° for 30 days, when

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[2] Appreciation is expressed to Eugene Herrling for making the photographs used in this manuscript, to Dr. J. H. Torrie for statistical counsel, and to Robert Raabe for making some of the local lesion counts.

[3] HOGGAN, I. A., and JOHNSON, J. A VIRUS OF CRUCIFERS AND OTHER HOSTS. Phytopathology 25: 640–644, illus. 1935.

[4] POUND, G. S. HORSERADISH MOSAIC. Jour. Agr. Res. 77: 97–114, illus. 1948.

[5] POUND, G. S., and WALKER, J. C. DIFFERENTIATION OF CERTAIN CRUCIFER VIRUSES BY THE USE OF TEMPERATURE AND HOST IMMUNITY REACTIONS. Jour. Agr. Res. 71: 255–278, illus. 1945.

[6] POUND, G. S., and WALKER, J. C. EFFECT OF AIR TEMPERATURE ON THE CONCENTRATION OF CERTAIN VIRUSES IN CABBAGE. Jour. Agr. Res. 71: 471–485 illus. 1945.

an assay was made for virus concentration in plants at 16° and 28°. During the summer of 1947 the plants were transplanted to the field. In October they were lifted and roots from each of the four groups were replanted to pots and placed at each of the four greenhouse temperatures mentioned above.

In preparing inoculum for an assay of virus concentration, a composite sample of 500 milligrams of tissue was selected from the youngest leaves of each pot of a given treatment. Only marginal leaf tissue from closely comparable leaves was used, care being exercised to avoid midrib tissue. The tissue was macerated in 10 cubic centimeters of distilled water until it was macroscopically homogeneous. The assay consisted of half-leaf inoculations to tobacco (*Nicotiana tabacum* L. var. Connecticut Havana No. 38) on which the virus produces only necrotic local lesions. Tobacco plants were evenly sprinkled with carborundum before the inoculum was applied with a glass spatula.

When only one comparison was to be made, two to five tobacco plants with three leaves each were used. When all possible comparisons of the four temperatures were desired, seven tobacco plants of three leaves each were used in an incomplete block design.[7] [8] The following arrangement was used in which horizontal rows represent tobacco plants and vertical columns represent leaf positions on these plants.

A	B	C	D	E	F	G
B	D	F	E	G	A	C
C	F	E	A	B	G	D

Since the four temperatures allowed only six different comparisons, one treatment was duplicated each time. It can be seen that each treatment occurred once at each leaf position and once on the same plant with each of the other treatments. Occasional uniformity

TABLE 1.—*Results of 3 uniformity trials to test the half-leaf carborundum method used in assaying virus concentration*

Hypothetical half-leaf comparisons	Average number of local lesions in 3 replicates in trial indicated		
	Trial 1 [1]	Trial 2 [1]	Trial 3 [1]
A {Left	59.3	83.0	84.3
A {Right	84.7	199.7	75.0
B {Left	39.3	141.3	64.7
B {Right	42.0	178.7	62.3
C {Left	39.0	149.0	63.0
C {Right	45.3	153.0	64.0
D {Left	41.0	192.3	125.7
D {Right	54.7	214.7	139.0
E {Left	39.7	163.0	109.3
E {Right	38.7	179.7	108.0
F {Left	47.0	139.3	93.0
F {Right	59.7	151.0	83.7
G {Left	49.0	97.0	54.3
G {Right	52.3	87.7	67.0

[1] No significant difference between treatments occurred in any of the trials.

[7] YOUDEN, W. J. USE OF INCOMPLETE BLOCK REPLICATIONS IN ESTIMATING TOBACCO-MOSAIC VIRUS. Boyce Thompson Inst. Contrib. 9: 41–48, illus. 1937.

[8] YOUDEN, W. J., and BEALE, H. P. A STATISTICAL STUDY OF THE LOCAL LESION METHOD FOR ESTIMATING TOBACCO MOSAIC VIRUS. Boyce Thompson Inst. Contrib. 6: 437–454, illus. 1934.

trials in which the same experimental design was used were run to assure the reliability of the half-leaf carborundum method. In these tests the same inoculum was applied to both halves of all leaves. Results of three such trials are given in table 1. It can be seen that in each of the three trials the half-leaf method was very reliable.

EXPERIMENTAL RESULTS

EFFECT OF TEMPERATURE ON SYMPTOMS AND LEAF MORPHOLOGY

When horseradish roots were forced in the greenhouse, symptoms appeared first at 28° and last at 16°. Initial symptoms consisted of a blotchy chlorotic mottle which sometimes was preceded by chlorotic vein clearing. Gradually, the symptoms at 28° and 24° became less intense and ultimately were completely masked. Leaves became dark green and showed no mottling and only rare necrotic streaking. At the same time symptoms at 20° and 16° became more intense, the blotchy mottle being replaced by a severe diffuse, yellow mottle and conspicuous necrotic ringing and streaking. When tops were removed and temperatures reversed, a corresponding complete reversal in symptom intensity ultimately occurred. When plants were taken from 28° and 16° and grown at the same temperature, they gradually became indistinguishable.

Field observations have shown a fernlike leaf to be very prevalent in horseradish. In the present studies this pinnatifid leaf type was found to be very prevalent at 16° C. but practically nonexistent at 28° (fig. 1). A perfect gradient existed from 28° to 16°, going from broadly laminate to pinnatifid leaves, respectively. When tops were removed and temperatures reversed, leaf type for the first few days was characteristic of the temperature at which the plants had grown before the temperature reversal (fig. 2). This was no doubt due to the fact that the leaf primordia for these early leaves were already initiated at the time of the temperature reversal. Sixty days after the temperature change, however, a complete reversal in leaf type had occurred, pinnatifid leaves being formed at 16° and laminate leaves at 28°. When plants from each temperature showing the characteristic leaf type were planted together in the field, they gradually became indistinguishable. When each group was returned to the different temperatures in the greenhouses, the same response to temperature occurred.

To ascertain if this fernlike leaf was correlated with virus concentration the following tests were made. Plants were grown at 28° and 16° C. until the respective leaf types were well established. The groups were divided into two parts each, the tops were removed, and temperatures were reversed on one-half of each group. Two weeks later both leaf types at both temperatures were present. Assays of virus concentrations were made between the two-leaf types at both 28° and 16°. No significant difference was found in the virus concentration between the two-leaf types at either temperature. However, fernlike leaves at 16° had a higher virus titer than laminate leaves at 28° but fernlike leaves at 28° had a lower virus titer than laminate leaves at 16°. Thus it appears that the temperature effect on leaf morphology is independent of that on virus concentration detailed in the following section.

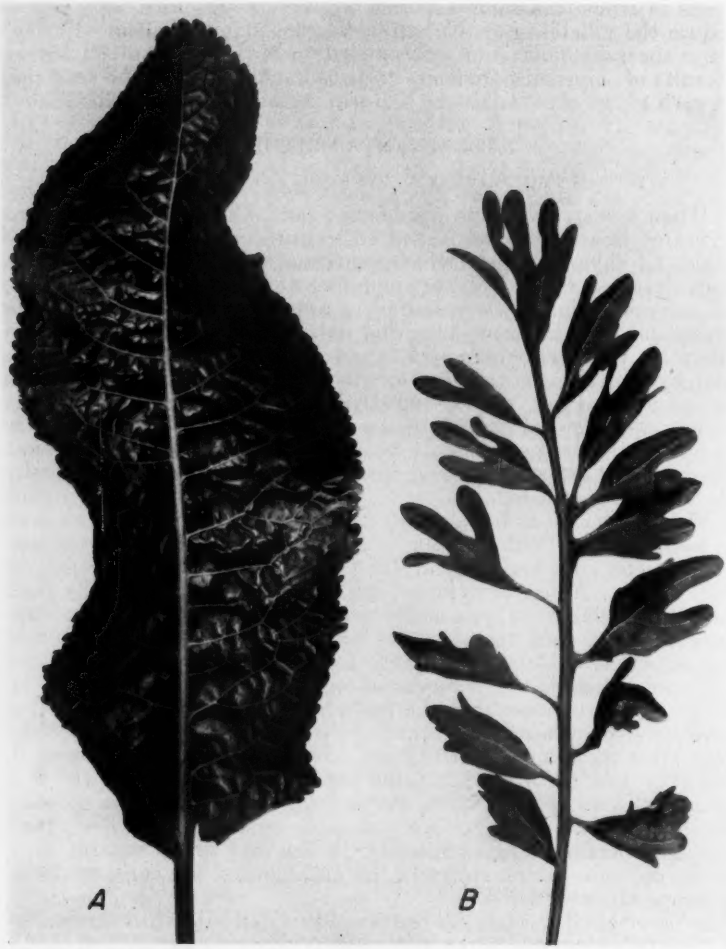

FIGURE 1.—Types of leaves produced on mosaic-infected horseradish at 28° C (*A*) and 16° C. (*B*).

EFFECT OF TEMPERATURE ON VIRUS CONCENTRATION IN HORSERADISH

Thirty days after the plants were placed at the different temperature levels in the spring of 1947, those at 16° and 28° C. were assayed for virus concentration. The following results were obtained.

	Average number of local lesions in half-leaf comparisons in 16 replications
16° C	37. 9
28° C	4. 9
Difference required for significance (19:1)	15. 7

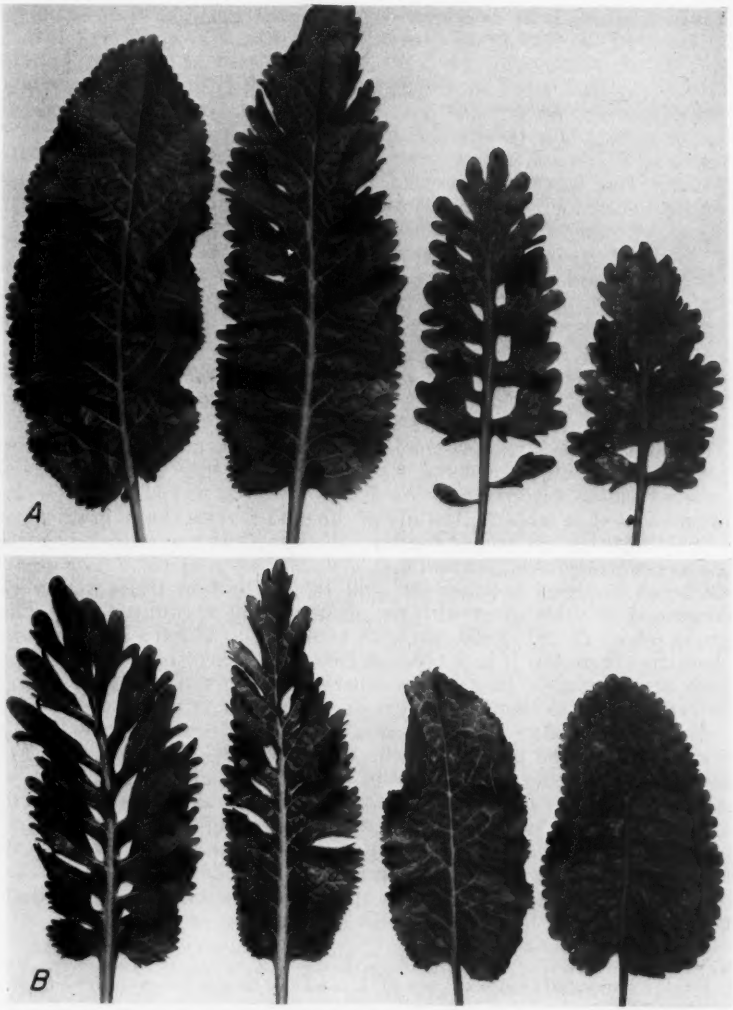

FIGURE 2.—Representative leaf types of mosaic-infected horseradish from plants grown at, left to right, 28°, 24°, 20°, 16°, respectively: *A*, from plants grown for 60 days at their respective temperatures; *B*, from plants grown for only 2 weeks at temperatures shown and prior to that for 60 days at, left to right, 16°, 20°, 24°, 28°, respectively.

It can be seen that in this single assay the virus concentration appeared to be much higher in plants grown at 16° than in those grown at 28°.

In October 1947, the plants were returned from the field and roots from each group were placed at the four different temperatures.

TABLE 2.—*Comparative concentration of horseradish mosaic virus in horseradish plants after growing for 60 days at 16°, 20°, 24°, 28° C., December 1947*

Half-leaf temperature comparisons (° C.)	Average number of local lesions in 3 replicates from inoculum of group [1]—				Total of 4 groups	*F* value for half-leaf comparisons [2]
	1	2	3	4		
28	2.7	2.3	1.0	0.7	6.7	12.2**
24	5.7	9.3	12.7	4.3	32.0	
28	5.0	3.7	2.7	2.3	13.7	24.1**
20	43.3	16.0	13.3	24.7	97.3	
28	4.0	1.3	1.7	1.0	8.0	80.2**
16	251.7	179.0	207.7	112.7	775.1	
24	4.3	3.0	10.7	4.3	22.3	8.7*
20	62.3	14.7	9.7	21.3	108.0	
24	9.3	5.7	11.7	5.0	31.7	72.6**
16	205.0	200.7	212.7	128.3	746.7	
20	78.3	12.3	11.3	5.7	107.6	28.7**
16	148.3	165.0	231.3	132.7	677.3	

[1] Groups 1, 2, 3, 4 correspond, respectively, to lots grown at 16°, 20°, 24°, and 28° C. during the spring of 1947 and in the field during the summer of 1947.
[2] *values are significant at the 5-percent level. **values are significant at the 1-percent level.

After 60 days, assays were made from all groups at all temperatures, each group being considered separately and being tested on seven tobacco plants according to the design given on p. 162. The results are presented in table 2. A study of the data reveals that a gradient of virus concentration existed between the temperatures, with extremely low concentrations at 28° and high concentrations at 16°. The greatest break occurred between 20° and 16°. The four trials were very consistent in their trend with no reversal to be accounted for. The group grown at 28° in the spring of 1947 (group 4) gave fewer lesions than the others but it is doubted if that was a carry-over effect of the high temperature. In another experiment the virus titer in young leaves at 16° was found to be greater than that in old leaves at 28°.

Immediately after the above assays were made, all top growth was removed from the plants and the pots from 16°, 20°, 24°, and 28° houses were transferred to houses of 28°, 24°, 20°, and 16°, respectively. Assays were made from the plants at 28° and 16° of groups 1, 2, 3, and 4 on the fifteenth, eighteenth, twenty-seventh, and twenty-ninth days, respectively, after reversal of temperatures. The results are given in table 3. It can be seen that even after only 15 days at the new temperatures the virus concentration of 16° was significantly higher than that at 28°. In all four groups this was the case.

TABLE 3.—*Comparative concentrations of horesradish mosaic virus in horseradish plants grown for 60 days at 16° and 28° C., and then for 2 to 4 weeks at 28° and 16°, respectively*

Temperature after reversal (°C.)	Average number of local lesions in 6 replicates from inoculum from group [1]—			
	1	2	3	4
16	73.0	89.0	173.5	109.3
28	34.8	19.2	5.5	32.1
L. S. D. (19:1)	16.5	30.1	32.6	28.9

[1] Assay from group 1 made 15 days after temperature reversal. Assay from group 2 made 18 days after temperature reversal. Assay from group 3 made 27 days after temperature reversal. Assay from group 4 made 29 days after temperature reversal.

Sixty days after the temperature reversal a further assay of virus concentration was made from plants growing at the 16° and 28° temperatures. In this assay, inoculum was prepared from the very youngest leaves of each group and also from the oldest leaves. The oldest leaves would have been formed soon after the temperature reversal. Because of a shortage of suitable assay plants groups 1 and 2 were combined and groups 3 and 4 were combined. The results, which are given in table 4, show that the virus was still in significantly higher concentration at 16° than at 28° but that the concentration in young leaves grown at 16° was considerably lower than in previous tests. In one case, the concentration in young leaves at 16° was significantly greater than that at 28° only at the 5-percent level of probability. No explanation can be given for this drop in concentration other than that the plants by that time were not in a very active state of growth. It is also notable that the virus concentrations from the combination of groups 3 and 4 were higher than from groups 1 and 2, but some of this difference could be accounted for in the difference between assay plants used.

TABLE 4.—*Comparative concentrations of horseradish mosaic virus in horseradish plants grown for 60 days at 16° and 28° C. and then for 60 days at 28° and 16°, respectively*

Temperature after reversal (° C.)	Average number of local lesions in 6 replicates for group and inoculum indicated			
	Groups 1 and 2 combined [1]		Groups 3 and 4 combined [1]	
	Inoculum from youngest leaves	Inoculum from oldest leaves	Inoculum from youngest leaves	Inoculum from oldest leaves
16	14.7	93.1	26.1	356.0
28	6.3	5.1	7.8	101.9
L. S. D. (19:1)	4.8	43.6	16.2	57.8

[1] For explanation of groupings see footnote of table 3.

It was thought that perhaps the lower concentration at 28° than at 16° might be due to the presence of an inhibitor in expressed sap from plants grown at 28°. To check this point an experiment was run in which inoculum from each temperature was tested in a series of dilutions with the idea that if an inhibitor were present one might neutralize its activity by dilutions low enough to leave the virus active. The results are given in table 5. It can be seen that this test gave no indication of the presence of an inhibitor.

As a further test for the presence of an inhibitor inoculum from plants grown at 28° was divided into two equal portions. One portion was treated with 10 percent Nuchar W adsorbent; the other was untreated. When the two inocula were compared in half-leaf inoculations, there was no significant difference in the number of local lesions produced. Thus, if an inhibitor was present it was not indicated by this test.

TABLE 5.—*Results of dilution of inoculum taken from plants grown at 28° and 16° C.*

[Dilutions compared in each case in half-leaf inoculations with undiluted control]

Extent of dilution	Average number of local lesions in 9 replicates			
	Inoculum from 28°		Inoculum from 16°	
	Control	Diluted	Control	Diluted
1–10	18.3	2.9	165.3	88.8
1–100	17.9	0	195.0	3.9
1–1,000	17.7	0	188.7	2.1

EFFECT OF TEMPERATURE ON THE CONCENTRATION OF THE HORSERADISH VIRUS IN RAPE

Horseradish viruses produce severe symptoms on plants of Dwarf Essex rape (*Brassica napus* L.).[9] To ascertain whether the virus in question showed a differential temperature response in rape the following experiment was performed. Forty plants of rape in the first true-leaf stage were inoculated with a composite inoculum from all horseradish plants under study. After 5 days' incubation at 22°, they were divided equally between the 16° and 28° houses. After 35 days, assays were made, comparing inocula from 16° and 28° plants both from youngest leaves (tenth leaf above cotyledons at 16°; twelfth leaf above cotyledons at 28°) and from old leaves (fifth leaf above cotyledons at both temperatures). The results, which are given in table 6, show that in young leaves the virus was in higher concentration at 28° than at 16° but in old leaves the reverse was true. In additional inoculations not shown in table 6 it was found that at both temperatures the concentration was greater in old leaves than in young leaves although significant only at the 5-percent level for the 28° plants. A much greater difference occurred between the young and old leaves at 16°.

TABLE 6.—*Comparative concentrations of horseradish virus in rape plants grown at 16° and 28° for 35 days prior to assay*

Temperature (°C.)	Average number of local lesions in 12 replicates from inoculum from—	
	Young leaves	Old leaves
16	41.8	234.3
28	75.5	112.2
L. S. D. (19:1)	12.2	35.0

EFFORTS TO INACTIVATE THE VIRUS IN HORSERADISH ROOTS AND PLANTS BY TEMPERATURE TREATMENTS

Since the thermal inactivation point of the horseradish virus was found to be slightly under 60° C. for 10 minutes, efforts were made to inactivate it in live roots by hot-water treatments and in growing plants by exposure to excessively high-air temperatures. Both experiments were unsuccessful, as shown by the data in table 7.

[9] See footnote 4, p. 161.

Top growth was burned badly by the high-air temperatures but terminal growing points were not killed and subsequent growth developed conspicuous symptoms. The first growth produced after the treated roots were planted also showed conspicuous symptoms.

TABLE 7.—*Results of hot water treatments of horseradish planting roots and hot air treatments of growing horseradish plants*

Time and temperature (°C.) of treatment	Number of roots or plants treated	Number of survivors	Number of survivors showing symptoms	Virus recovery test from survivors
Hot water:				
Untreated	20	20	20	Positive.
10 minutes at 65°	4	0		
10 minutes at 60°	10	0		
10 minutes at 55°	10	0		
20 minutes at 50°	[1] 8	4	4	Do.
30 minutes at 50°	10	0		
30 minutes at 45°	8	8	8	Do.
120 minutes at 45°	10	3	3	Do.
45 minutes at 40°	4	4	4	Do.
240 minutes at 40°	10	2	2	Do.
Hot air:				
48 hours at 50°	7	7	7	Do.
72 hours at 50°	7	7	7	Do.
96 hours at 50°	7	7	7	Do.
120 hours at 50°	7	7	7	Do.

[1] Roots of three-fourths inch diameter were used for this test. In all other tests roots of one-half inch diameter were used.

DISCUSSION

The data presented herein are interpreted as indicating different levels of virus concentration in horseradish plants growing at different temperatures. However, the possibility cannot be overlooked that a virus inhibitor may be present in horseradish and may occur more at one temperature than at another. Although two tests failed to indicate any inhibitor the possibility has not been fully explored.

The development of more conspicuous and more severe symptoms of horseradish mosaic in the field in late fall is explainable by results reported in this paper. At high air temperatures symptoms are masked and the concentration of the virus is relatively low. At low temperatures symptoms are persistent and virus concentration is relatively high. The author has never observed horseradish growing in areas where average air temperatures would reach or surpass 28° C. (e. g., the East St. Louis area), but in Wisconsin and western Washington it has been observed that symptoms are most marked late in the fall. It would not be surprising if infected horseradish growing in southern areas would appear perfectly healthy in midsummer but quite diseased in late fall. This would be the case, however, only if other strains of horseradish mosaic virus showed the same temperature response as the one studied in this report.

The correlation of symptom severity with virus concentration in horseradish is similar to that reported for certain cabbage strains of turnip virus 1[10] except that symptoms on cabbage were more pronounced and viruses were in higher concentrations at high temperatures than at low temperatures. The concentration of the cabbage

[10] See footnote 6, p. 161.

strains was not determined in any other host nor was that of the horseradish virus determined in cabbage. The different temperature responses shown by the horseradish strain and the cabbage strains indicate that all strains of turnip virus 1 are not favored by high air temperatures on all hosts as suggested by Pound and Walker.[11]

The fact that the horseradish virus occurred in higher concentration at 28° than at 16° in young leaves of rape but that the reverse occurred in old leaves further indicates that the effect of temperature depends upon the specific host-virus complex and may be due to the influence of the environal factor on the host, the virus, or both. It also indicates that in studies of physical properties where virus concentration is a limiting factor, one should give prime consideration to age of tissue involved as well as temperature at which the plants were grown.

No explanation is offered for the factors involved in the temperature effect on leaf morphology. Data presented herein seem to indicate that the reaction is independent of virus activity. This is further borne out by the fact that a horseradish clone from which no virus has been isolated and which has not become infected upon inoculation also develops the fernlike leaves. It is possible that temperature affects the physiology of the plant relative to some growth regulating substance.

SUMMARY

A virus causing horseradish mosaic was found to occur in much higher concentration in horseradish grown in greenhouses at 16° C. than at 28°. This was also true for old leaves of rape plants infected with the horseradish virus, but for young leaves of rape the concentration was higher at 28°.

When horseradish plants growing at different temperatures and showing a gradient of virus concentration were reversed in position, the plants being moved from 16° to 28°, etc., a corresponding reversal in virus concentration occurred.

A marked temperature effect on leaf morphology of horseradish was also recorded. At high temperatures, the prevailing leaf type was broadly laminate. At low temperatures leaves were exceedingly pinnatifid, often being reduced to extreme fernleaf structures. At intermediate temperatures intermediate types occurred. A reversal of temperatures resulted in a corresponding reversal in leaf type. The temperature effect on leaf type is thought to be independent of that on virus concentration.

Efforts to free horseradish roots and plants from the virus by heat treatments in hot water and hot air, respectively, were unsuccessful.

[11] See footnote 5, p. 161.

BREEDING LOW-NICOTINE TOBACCO[1]

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INTRODUCTION

In 1933 the Kentucky Agricultural Experiment Station received from J. B. Hutson, of the United States Department of Agriculture, then stationed in Germany, seven lots of Havana and Cuba cigar tobacco varieties low to very low in nicotine content. These seeds had been obtained from Dr. Erwin Baur, director of the Kaiser Wilhelm Institute. So far as the writer was aware, there were at that time no varieties of cigarette or pipe tobacco of low nicotine content. It was decided, therefore, to develop a variety or varieties of cigarette tobacco of low nicotine content to meet a possible demand for such a tobacco as a health measure. It was further decided that this variety should carry the recessive color factors of burley because burley is a light-colored tobacco when cured and light color is commonly associated in the mind of the public with mildness.

MATERIALS AND METHODS

Three strains of Cuba, designated 30/33, 31/33, and 32/33, each listed as very low in nicotine, were used as the source of the low-nicotine character. In 1934, analyses of these varieties showed that they contained 0.19, 0.05, and 0.11 percent nicotine, and in 1935, 0.18, 0.22, and 0.21 percent, respectively. These varieties were crossed with Ky 5, Ky 7, Ky 14, and Ky 16—all black-root-rot-resistant varieties of burley tobacco. In 1934 these varieties analyzed 2.18, 2.10, 1.37, and 2.18 percent nicotine, respectively.

All nicotine determinations were made on leaves from the middle of field-grown plants as these leaves are usually the highest in nicotine in tobacco harvested and cured like burley. During the past several years the tobacco has been grown on more fertile soil than in the early years, and the nicotine content of Ky 16, used as a control, has been higher. From 1934 to 1942 the nicotine determinations were made by members of the Department of Chemistry of the experiment station, who used the method of the Association of Official Agricultural Chemists (1, *p.* 64).[2] In 1943 and 1944 Markwood's (*10*) green disk method was used and determinations were made by E. M. Johnson. Since then the determinations have been made under the

[1] Received for publication May 17, 1948.

[2] Italic numbers in parentheses refer to Literature Cited, p. 180.

direction of R. B. Griffith who has used a rapid modification of the A. O. A. C. method (*6*). In 1947 determinations were made by the method of Bowen and Barthel (*3*).

EXPERIMENTAL RESULTS

CROSSES

The F_1 generation of the dark cigar tobacco by the light-colored burley was all dark, as would be expected. A single determination of nicotine in an F_1 30/33 × Ky 7 showed that it had 3.18 percent when Ky 16 had 1.73 percent and the low-nicotine parent had 0.22 percent. This suggests that the high-nicotine factor is completely dominant over low nicotine. The difference in nicotine content between the F_1 hybrid and Ky 16 is not significant.

The F_2 generation segregated with respect to plant color, producing approximately 15 dark plants to 1 light-colored plant. There was also segregation with respect to nicotine content, with a range from plants with no nicotine to 2.82 percent in a total of 345 burley-colored plants tested (table 1).

TABLE 1.—Nicotine content of burley-colored F_2 plants from low-nicotine cigar tobaccos crossed with burley, 1935

Nicotine (percent)	31/33×Ky 5	30/33×Ky 7	31/33×Ky 14	30/33×Ky 16	32/33×Ky 14	Total
0	2	3	4	2	14	25
.1	14	11	20	10	11	66
.5	9	20	23	26	21	99
1.0	22	17	22	18	12	91
1.5	11	15	4	6	3	39
2.0	10	8	5	2	0	25
Total plants	68	74	78	64	61	345

There appears to be no sharp segregation into low-nicotine and high-nicotine content plants as claimed by Hackbarth and Sengbusch (*9*), who concluded that the nicotine content of tobacco is dependent upon a single factor pair. However, these results do not disprove their contention, for with as variable a factor as nicotine, considerable variation might occur within any one class so that the three classes, homozygous low, heterozygous, and homozygous high, might run together. There are also the complicating factors of nicotine and nornicotine. It will be shown later that in some segregates much of the nicotine seems to change to nornicotine, some of which is determined as nicotine by the A. O. A. C. method.

In 1936 the F_3 generation of some of the very low nicotine plants from the previous year were grown. Of these a few were selected for plant type and nicotine determinations made. The results are given in table 2.

It is evident that burley-colored plants low in nicotine in the F_2 generation do not always produce plants of equally low nicotine content in the F_3 generation; but the F_3 plants from selected low-nicotine F_2 plants are sometimes all relatively low in nicotine and on an average are much lower than burley.

TABLE 2.—*Nicotine content of F_2 plants and of F_3 burleylike plants of low nicotine × high nicotine hybrids*

F_3 generation of cross—	Nicotine content of F_2 plants	F_3 plants	Range in nicotine content of F_3 plants	Average nicotine content of F_3 plants
	Percent	*Number*	*Percent*	*Percent*
30/33×Ky 7	0.07	4	0.24–.30	0.27
31/33×Ky 14	.01	1	.02	.02
Do	.06	5	1.00–1.56	1.23
Do	.07	5	.32–.98	.70
32/33×Ky 14	.03	5	.46–1.77	1.34
Do	.06	7	.07–1.56	.66
30/33×Ky 7	.01	7	.03–1.11	.60
Do	0	6	1.15–1.78	1.45
32/33×Ky 14	.02	7	.16–.98	.48
Do	.02	9	.52–3.60	1.78
Do	.02	14	.13–2.45	1.26
Do	.02	5	.28–.46	.38

In 1937 determinations of nicotine in seven F_4 plants of a strain from a 0.02-percent F_3 plant showed all to contain 0.05 percent or less; progeny of a 0.07-percent plant contained less than 0.3 percent; progeny of a 0.02-percent plant contained 0.45 percent or less; while in some groups of plants from very low nicotine parents the nicotine content ranged from 0.20 to 2.45 percent. The results seemed to show, however, that extremely low nicotine content strains of large-leafed tobacco having the color of burley could be isolated.

BACKCROSSES

In 1936 it became evident that it would not be possible to select desirable types of tobacco without further backcrossing. Therefore, several backcrosses were made in which Ky 16 was used as the pollen parent and progeny of several very low nicotine plants were used as the seed parents. There was no assurance, however, that the seed parents were all low in nicotine. The next year a good type plant of the F_2 backcross, later shown to contain 1.08 percent nicotine, was crossed with several F_3 32/33×Ky 14, the parent of which tested 0.02 percent nicotine, and with F_4 31/33×Ky 14, of which seven were tested for nicotine and the highest was found to contain 0.05 percent. Another F_2 segregate containing 3.25 percent nicotine was also used (table 3).

TABLE 3.—*Result of crossing F_2 segregates of low-nicotine burleylike plants × Ky 16, with low-nicotine plants*

Nicotine content of parents (percent)	Plants tested	Nicotine content	
		Range	Average
	Number	*Percent*	*Percent*
0.03×1.08	1	0.05	
0.01×1.08	19	.01–.60	0.27
0.01×1.08	19	.01–.58	.06
0.06×1.08	13	.02–1.04	.36
0.13×1.08	13	.01–1.21	.21
0.07×3.25	17	.05–2.09	1.49
Progeny of 1.08 percent plant used in above crosses	15	.29–.89	.49
0.45-percent —— selection of 1.08-percent plant	6	.71–1.05	.88
0.33-percent —— selection of 1.08-percent plant	3	.45–.94	.74

The results of these backcrosses indicate that a plant testing as high as 1.08 percent, when crossed with established low-nicotine strains, produces progeny low in nicotine; but where a high-nicotine plant possibly carrying a factor or factors for low nicotine was used as one parent the F_2 progeny ranged from 0.05 percent to 2.09 percent, with an average of 1.49 percent nicotine.

In 1939, F_3 progeny of plants crossed with the 1.08-percent nicotine plant (table 3) were grown from F_2 plants testing 0.06 percent nicotine or less. Of 52 F_3 plants tested, the nicotine content ranged from 0.01 percent to 2.41, with an average of 0.34 percent. Progenies of several plants were all below 0.1 percent, indicating that very low nicotine strains could be selected following backcrossing with a high-nicotine parent.

In 1940, eight F_4 progeny of a 0.02-percent F_3 plant averaged 0.025 percent nicotine. A selection with 0.9 percent nicotine from the 0.07- × 3.25-percent nicotine cross (table 3, line 6) gave progeny (seven tested) ranging from 1.06 to 1.59 percent nicotine, with an averge of 1.29 percent, a figure definitely below the nicotine content of burley tobacco grown under the same conditions.

In 1940, the F_2 generation of a second backcross with Ky 16 on a 0.02-percent plant was grown. Nicotine content ranged from 1.11–4.50 in 26 plants tested. While the majority were high, 4 contained less than 1.75 percent nicotine. Four F_3 plants of the plant testing 1.11 ranged from 0.71 to 1.20 percent nicotine.

In 1942, plants from 10 low-nicotine selections from the F_5 generation of crosses listed in table 3 and F_3 second backcrosses of low-nicotine plants of these with Ky 16 were grown. In all, 80 plants were tested for nicotine content. Nicotine ranged from 0.01 percent to 0.90 percent, when Ky 16 grown under the same conditions contained 3.8 percent. The average nicotine content of the 80 plants was 0.246 percent, showing that low-nicotine strains could be isolated after 2 or 3 crosses with burley.

NICOTINE CONTENT OF CROPS OF LOW-NICOTINE TOBACCO

Four small commercial crops of low-nicotine burleylike tobacco were studied for nicotine content in 1945. They were grown on fertile soil and all made rapid, vigorous growth. Nicotine determinations were made of individual seed plants and the nicotine content of a composite sample of a middle leaf from each of 25 topped and suckered plants was determined. A few upper leaves had been stripped from the seed plants, and the plants had been suckered. Samples were collected at the time the remainder of the crop was cut, taking 4 leaves from near the center of the plant from each seed plant and 1 similarly placed leaf from each of 25 topped and suckered plants. Nicotine determinations were made by the quick method of Griffith and Jeffrey (*6*) after the tobacco was air-cured. Distillations were made from MgO and from NaOH–NaCl.

Three of the strains appear to have been low-nicotine strains (table 4), while one (S 2) apparently contained an occasional plant of higher nicotine content that increased the average nicotine content of this strain above that of the others.

TABLE 4.—*Alkaloid determinations of 4 crops of low-nicotine tobacco, 1945*

Strain	Seed plants	Range in nicotine content determined with MgO	Average	Range in nicotine content determined with NaOH-NaCl	Average
	Number	*Percent*	*Percent*	*Percent*	*Percent*
S 1	25	0.002–0.435	0.109	0.002–0.862	0.231
S 2	24	.001–1.375	.498	.017–1.414	.670
S 3	25	.031– .352	.130	.055– .932	.402
S 4	24	.051– .334	.140	.059– .874	.394
S 1	Composite of 25 topped plants.	.035		.276	
S 2		.675		.875	
S 3		.173		.489	
S 4		.125		.427	

A half-acre crop of another strain was grown in 1946 for Dr. H. B. Haag, of the University of Virginia, for his pharmacological studies with low-nicotine tobacco. Dr. Haag reported that after redrying, a thieved sample from a hogshead gave, on analysis, 0.08 percent nicotine, 0.06 percent nornicotine, and 0.14 percent total alkaloids. A previous half-acre crop of low-nicotine tobacco raised for Dr. Haag for studies on the role of nicotine in the cigarette habit contained 0.23 percent nicotine (*4*).

There seems to be no doubt from these results that commercial crops of low-nicotine tobacco very low in nicotine content can be grown under ordinary field conditions.

NICOTINE AND NORNICOTINE CONTENT OF LOW-NICOTINE TOBACCO

In 1945 and 1946 a modification of the A. O. A. C. method was used for nicotine determinations with the quick distillation apparatus of Griffith and Jeffrey (*6*). Distillations were made with both MgO and NaOH-NaCl. There is a marked difference in alkaloid content as determined by these methods. So far as Griffith has been able to determine, MgO distillation gives a high value for nicotine since 35 to 40 percent of the nornicotine present in pure or mixed solutions is obtained in the distillate. The NaOH-NaCl concentrations used gave the total steam volatile alkaloids as nicotine. The difference between the MgO and the NaOH-NaCl results gives an approximation of alkaloids other than nicotine, usually assumed to be nornicotine The A. O. A. C. method as used in the early studies (*1, p. 64*), according to unpublished work of Griffith, measures nicotine and from 10 to 30 percent of nornicotine or other compounds and so is not accurate for low-nicotine varieties, some of which appear to contain considerable nornicotine. The present A. O. A. C. (*2, p. 74*) method measures nicotine plus nornicotine and so is not as satisfactory for low-nicotine varieties as the older method.

If the MgO values of all low-nicotine selections are arranged in order, from low to high, and divided by the respective NaOH-NaCl values to determine percentage of alkaloids that is largely nicotine, the figure rises from a very low one (less than 7 percent at the 0–0.01 level) to over 90 percent at the 3-percent level [3] (table 5). That is,

[3] This relationship was called to the writer's attention by Dr. R. N. Jeffrey.

when nicotine is very low the percentage of total alkaloids that is nicotine is correspondingly low, while as the MgO value rises it becomes a greater and greater proportion of the total alkaloids until in ordinary tobacco 85 to 95 percent of the total alkaloid is nicotine or at least is distilled over from MgO.

TABLE 5.—$\frac{MgO}{NaOH-NaCl}$ *values (approximate percent of total alkaloids that is nicotine) in low- and high-nicotine content tobaccos in 1945 and 1946*

MgO value	1945		1946	
	Number of plants	MgO / NaOH−NaCl	Number of plants	MgO / NaOH−NaCl
0. − .01	13	0.0660	9	0.0085
.01− .05	14	.2189	23	.0868
.05− .10	26	.3074	13	.1298
.1 − .5	103	.4641	39	.2530
.5 −1.0	44	.5903	14	.6328
1.0 −1.5	27	.8552	10	.8676
1.5 −2.0	27	.8995	2	.8759
2.0 −2.5	8	.8582	--------	----------
2.5 −3.0	2	.8665	--------	----------
3.0 −3.5	6	.9392	2	.8778
3.5 −4.0	1	.9411	--------	----------
4.0 −5.5	3	.9399	--------	----------

Table 5 also shows that the MgO/NaOH-NaCl value, or approximate percent of total alkaloids that is nicotine, in low-nicotine tobaccos was decidedly higher in 1945 than in 1946.

While in general the proportion of nicotine to total alkaloids in the tobaccos studied increases as the total alkaloid content increases (table 5), this does not mean that strains of tobacco cannot be selected that differ markedly in the relation of nicotine to total alkaloids at any given level of the latter. In the progeny of a cross of a 0.02-percent nicotine content burleylike plant × Turkish (Basma) backcrossed with a 0.02 percent burleylike plant, an F_2 of the backcross was selected that tested 0.43 percent nicotine when plants of Ky 16 at the same stage of growth tested 2 percent. The F_3 progeny of this plant ranged in MgO value from 0.082 to 1.007 (average of 24 plants, 0.412), and in NaOH-NaCl value from 0.193 to 1.131 (average of 24 plants, 0.612). The F_4 plants of selected F_3's showed distinct segregation as far as the MgO value was concerned, while the NaOH-NaCl value for the group was remarkably uniform, ranging from 0.38 to 2.23 percent, with 53 of the 75 plants tested lying between 0.85 and 1.8 percent. The range and average of the MgO value, the average of the NaOH-NaCl value, and the ratio between the two values are given in table 6.

In this table the MgO/NaOH-NaCl values have been arranged in descending order, indicating that strains have been isolated having an NaOH-NaCl or total alkaloid content of about 1.5 percent but an MgO value or approximate nicotine content ranging from 94 percent of the total alkaloid content to only 16.6 percent.

TABLE 6.—*Alkaloid content of strains of tobacco resulting from a cross of low-nicotine burleylike tobacco with Turkish tobacco, and Ky 16, as determined by distillation with MgO and NaOH-NaCl, 1946 crop*

Plants (number)	MgO value		NaOH-NaCl average	MgO/NaOH-NaCl
	Range	Average		
	Percent	*Percent*	*Percent*	*Percent*
7	0.58 –1.17	0.89	0.94	0.9408
7	.91 –1.85	1.44	1.59	.9034
3	.19 – .64	.47	.58	.8057
8	.401–1.58	1.06	1.33	.8014
7	.50 –1.10	.77	.98	.7779
4	.18 – .93	.66	.87	.7557
6	.37 –1.50	.94	1.64	.5705
7	.32 – .67	.52	1.21	.4329
9	.11 – .90	.51	1.29	.3945
7	.29 – .71	.49	1.29	.3837
6	.13 – .65	.31	.81	.3830
3	.14 – .27	.22	1.32	.1661
Ky 16	3.08 –3.74	3.30	3.68	.8951

While these results are based on small numbers, they give proof that strains of tobacco closely similar to burley in general characteristics can be isolated that have a total alkaloid content of approximately 1 to 1.5 percent when ordinary burley has about 3.5 percent. The results suggest further that in this range strains can be isolated with a large proportion of the alkaloid in the form of nicotine, while other strains appear to have a large proportion of the alkaloid as nornicotine. Strains of cigarette tobacco that are not likely to produce over 1.5 to 2 percent nicotine under conditions that result in 3.5 percent or more nicotine in ordinary burley may prove of great value to the trade.

In 1947 strains derived either directly from the low nicotine × burley backcrosses or from crosses of low nicotine burleylike plants with Turkish tobacco were grown for nicotine determination. The plants from which samples were to be collected were bagged for seed but were kept suckered. Leaf samples were collected from the middle of the plants, at normal cutting time, and air-cured. Nicotine and nornicotine determinations were made by the Bowen-Barthel method (*3*). Ten plants of Ky 16 and 15 plants each of 19 low nicotine strains were saved for analysis.

The majority of the strains appeared to be fairly homozygous with respect to nicotine and nornicotine. In table 7 the strains are arranged in order of increasing nicotine content. Ten strains proved to be very low in nicotine, the highest averaging 0.05 percent. The other 9 strains ranged from a low average of 0.319 percent nicotine to 0.813. One of the low nicotine strains (range 0 to 0.042) had a range of nornicotine from 0.100 to 1.61 with an average of 0.670. This furnishes the best evidence so far obtained in these studies that strains might be isolated with a relatively high total alkaloid content (about 1 percent) nearly all of which is nornicotine. There is abundant evidence that strains can be isolated with very low nicotine as well as low total alkaloid content.

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TABLE 7.—*Nicotine and nornicotine content of 19 strains of low-nicotine tobacco and Ky 16, grown in 1947, as determined by the Bowen-Barthel method*

Nornicotine		Nicotine		Total Alkaloids		Nicotine
Range	Average	Range	Average	Range	Average	Total alkaloids
Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	
0.005- .058	0.028	0 - .006	0.0004	0.005- .064	0.028	.01
.242- .648	.384	0 - .015	.003	.242- .648	.388	.008
.019- .257	.138	0 - .069	.006	.083- .257	.144	.04
.026- .268	.164	0 - .018	.007	.034- .268	.162	.04
.001- .062	.031	0 - .027	.010	.001- .077	.041	.24
.211- .655	.412	0 - .039	.010	.230- .681	.422	.02
.100-1.61	.670	0 - .042	.013	.116- .64	.683	.02
0 - .299	.049	0 - .065	.014	0 - .305	.063	.22
.195- .582	.379	0 - .349	.044	.207- .544	.422	.10
.046- .201	.128	0 - .254	.050	.098- .300	.179	.28
.008- .098	.052	.256- .447	.319	.298- .506	.371	.86
.042- .373	.144	.165- .615	.391	.337- .832	.535	.73
.035- .373	.154	.137- .880	.527	.280-1.075	.654	.81
.025- .133	.074	.447- .705	.589	.525- .830	.656	.89
.070- .253	.126	.111- .855	.617	.364-1.605	.810	.76
.006- .339	.139	.118- .905	.642	.181-1.174	.777	.83
.043- .248	.106	.381- .946	.663	.556- .995	.770	.86
.062- .159	.122	.475- .883	.690	.690-1.022	.811	.85
.040- .317	.135	.457-1.690	.813	.581-1.852	.944	.86
[1].106- .472	.290	1.08 -1.75	1.457	1.38-1.96	1.760	.83

[1] Ky.16

When the average nicotine content of each strain was divided by the average total alkaloid content the 19 strains were again divided sharply into 2 groups, one with 28 percent or less of the total alkaloids in the form of nicotine and the other with 73 percent or more of the total alkaloids as nicotine. All of the latter group contained over 0.3 percent average nicotine while all of the former group contained an average of 0.05 percent or less. These low-nicotine strains could all be considered primarily nornicotine strains as most of the alkaloid appears to be nornicotine. Four of the strains in the low-nicotine group contained a higher average total alkaloid content than the lowest of the higher nicotine group. These results suggest that in breeding for low-nicotine varieties of tobacco, 2 sets of factors are concerned: (1) those that control total alkaloid production and (2) those that control the change of nicotine in the leaf to nornicotine or other products of nicotine. It is probably desirable to have varieties in which both sets of factors are operating, if the desired product is to be uniformly low in nicotine year after year. If complete dependence were placed on low total alkaloids these would undoubtedly vary from year to year and the nicotine content might be higher than desirable following, perhaps, a dry year. Moreover, if a factor is operating to change nicotine to some other product, the nicotine content of the tobacco will have much more opportunity to remain constant from year to year.

DISCUSSION

The object in developing a low-nicotine cigarette tobacco was to meet a possible demand for tobacco so low in nicotine that it could be smoked, without harmful effects from nicotine, by people who are sensitive to this alkaloid.

As to the immediate effects of nicotine on the smoker, there are at least two: an increase in blood pressure resulting from absorption of nicotine into the blood stream (*7*) and irritation of the mucous membranes with which the smoke comes in contact (*5*). Haag and Larson (*8*) in preliminary tests showed that—

> smoke from the low nicotine cigarettes on an average produced effects comparable to those observed after the smoking of nicotine-free cigarettes, both types of cigarettes evoking circulatory responses, markedly less than those effected by ordinary cigarettes.

Smoke from cigarettes made from low-nicotine tobacco was inhaled by persons with very sensitive throats with only the slightest feeling of irritation, smoke from a low-nicotine cigar with wrapper and binder of the usual type caused distinct but mild irritation, while smoke from an ordinary cigarette caused immediate coughing and left the throat very uncomfortable for several minutes. Cigarettes prepared from low-nicotine tobacco to which about 2 percent nicotine malate had been added (*5*) affected sensitive throats in exactly the same way as an ordinary cigarette. There seems to be little doubt that the immediate irritation of the throat by cigarette smoke is almost entirely caused by its nicotine content.

While the principal object of the present study was to produce a burley-colored cigarette tobacco of low nicotine content, yet the results have contributed something to the knowledge of the inheritance of nicotine-controlling factors. It seems certain, as has been pointed out by others, that high nicotine is dominant over low nicotine in F_1 crosses. In F_2 crosses the problem of classifying the individual plants is complicated by methods of nicotine determination. The A.O.A.C. (*1*, *2*) method is adapted to high-nicotine varieties in which most of the alkaloid is nicotine so that in the early studies not only nicotine but but nornicotine and perhaps other substances were reported as nicotine. While this did not delay work on selection for low nicotine, it undoubtedly contributed to confusion in understanding results from the genetic standpoint. It is obvious that in the low-nicotine tobaccos the inheritance of at least two sets of factors are involved, those concerned with the rate of nicotine production and those that control conversion of nicotine to nornicotine or other products. If the original alkaloid produced is nicotine, then there must be control mechanisms not only over the rate of nicotine production but also over its conversion to nornicotine, or other products.

The present study has demonstrated that the ability to produce only a small amount of nicotine and a low total alkaloid content may be transferred from low-nicotine cigar tobacco to a burleylike variety of tobacco. Assuming that nicotine is the alkaloid produced by the roots of these strains, then in some strains of low-nicotine tobacco there must be a mechanism for changing nicotine to some other product, presumably nornicotine, while, in other strains this ability occurs only to a very slight extent. If the ability to convert a considerable porportion of nicotine to some other product can be transferred to ordinary varieties of high-nicotine content tobacco without disturbing the variety too much otherwise, it is possible that the nicotine content of ordinary tobaccos might be kept at a more desirable level year after year.

The low-nicotine burleylike varieties of tobacco described herein have been recognized by the United States Department of Agriculture (*11*) as a subtype of burley and have been designated Type 31-V.

SUMMARY

Varieties of burleylike tobacco of very low nicotine content have been developed by crossing and backcrossing low-nicotine cigar tobacco with burley. The low-nicotine burleylike tobacco has been recognized by the United States Department of Agriculture as a subtype of burley and has been designated Type 31–V.

First generation crosses between burley and low-nicotine cigar tobacco were high in nicotine. In the F_2 generation there was segregation with respect to nicotine content. The percentage of nicotine ranged from 0 to 2.82, with no sharp line between low- and high-nicotine content. This may have been because both nicotine and nornicotine were reported as nicotine. Progeny of some low-nicotine F_2 plants produced only low-nicotine plants, but this was not always the case.

Commercial crops have been grown with an average nicotine content below 0.10.

In general, when the nicotine content of segregates is very low the percentage of total alkaloids that is nicotine is correspondingly low, while as the nicotine content rises the percentage of total alkaloids that is nicotine also rises until, in ordinary burley tobacco, 85 to 95 percent of the total alkaloid is nicotine. However, evidence was obtained that strains could be isolated with about 1 percent total alkaloid nearly all of which was nornicotine.

When the average nicotine content of low-nicotine strains was divided by the average total alkaloid content 19 strains were divided sharply into 2 groups; one with 28 percent or less of the total alkaloid in the form of nicotine, and the other with 73 percent or more of the total alkaloid as nicotine.

There appear to be two sets of factors controlling the inheritance of nicotine and nornicotine: those that control total alkaloid production, and those that control the change of nicotine in the leaf to nornicotine.

The immediate irritation of the throat by cigarette smoke appears to be caused almost entirely by its nicotine content.

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EFFECT OF HEAT ON BLACK ROT AND KEEPING QUALITY OF SWEETPOTATOES[1]

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INTRODUCTION

Of the many diseases that affect sweetpotatoes (*Ipomoea batatas* (L.) Lam.) in storage, black rot, caused by *Endoconidiphora fimbriata* (Ell. and Halst.) Davidson, probably ranks second in destructiveness only to soft rot caused by *Rhizopus* spp. Very little evidence of black rot may be present at digging time, but a large proportion of the roots may show the disease at the conclusion of storage. Black rot is also a field and seedbed disease, and considerable effort is expended to control it in each of its three phases. The control methods thus far developed for seed potatoes are surface sterilization with mercuric chloride or some similar treatment, careful building up of disease-free seed stock, and rotation of crops. No satisfactory chemical treatment has been devised to prevent the development of black rot in storage or in transit immediately after digging. Discarding visibly affected potatoes does not give control. Washed, freshly dug sweetpotatoes from crops infected with black rot are very often badly spotted by the time they reach the market. A simple and effective method of controlling this disease would be a boon to farmers as well as to storage operators.

Lauritzen [2] determined the range of temperature at which the black rot fungus will grow on culture media to be 49° to 94° F. and showed that infection of sweetpotatoes was possible within about the same range. Since previous experience of the present writers had demonstrated that sweetpotatoes can stand 110° for at least 3 days, the studies reported herein were made to determine the effect of heat treatments on the development of black rot in harvested sweetpotatoes. However, because a treatment that controlled black rot would be of relatively little value if it increased the amount of other rots in the roots, caused excessive loss of weight, reduced the culinary quality, or decreased the sprouting capacity, the effects of the treatments on these factors also had to be evaluated both when black rot was present in the field and when it was absent. In addition, the effect of washing the potatoes on the effectiveness of the treatments had to be taken into consideration.

[1] Received for publication July 22, 1948.

[2] LAURITZEN, J. I. INFECTION AND TEMPERATURE RELATIONS OF BLACK ROT OF SWEETPOTATOES IN STORAGE. Jour. Agr. Res. 33: 663–676, illus. 1926.

MATERIAL AND METHODS

Orange Little Stem and Maryland Golden sweetpotatoes were purchased from nearby growers. Except in test 55, all lots were placed under treatment the day they were dug. The material for test 55 was obtained after it had been at a packing shed for an undetermined period. All treatments in tests 52, 55, and 56 had three replicates, but the treatments in tests 53 and 54 were not replicated. Each sample consisted of approximately one-half bushel of sweetpotatoes except in test 54 for which a peck was used.

Three insulated rooms kept, respectively, at approximately 110° F., 85°, and 55° were used. In the 85° and 55° rooms the temperature was held within ±3°, but in the 110° room it ranged from 94° to 114° (fig. 1). The relative humidity was about 80 percent in the 85° and

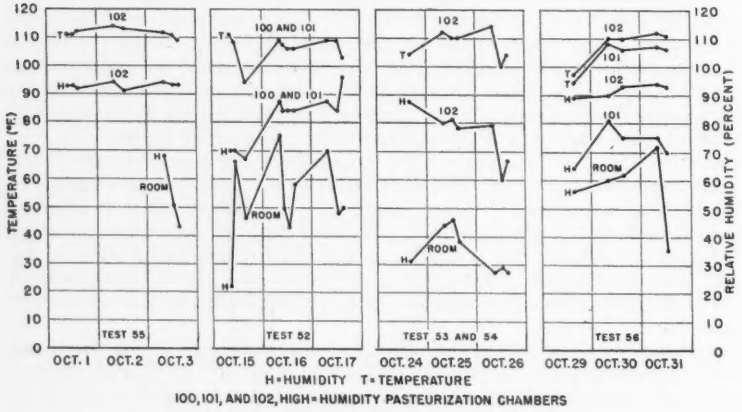

FIGURE 1.—Temperature and relative humidity in the pasteurization room and chambers during the several tests.

55° rooms. In the 110° room two humidity conditions were maintained. In three metal chambers (100, 101, and 102) having a capacity of twelve ½-bushel baskets each and equipped with fans to circulate the air over shallow pans of water, the atmospheric humidity was kept as near 85 percent as possible by regulating the area of exposed water and the rate of air movement over it. Outside the chambers the atmospheric humidity was in general kept at 70 percent or lower by use of anhydrous calcium chloride in shallow pans. The 110° room was designated the pasteurizing room; the 85° room, the curing room; and the 55° room, the storage room.

The term "pasteurization" is used to mean the holding of the sweetpotatoes at 110° F. for various lengths of time. Different lots were pasteurized for 1, 2, or 3 days, some in the chambers (high humidity) and others outside (low humidity).

Curing consisted in holding the sweetpotatoes in the 85° F. room at about 80 percent humidity for 3 or 10 days with or without previous pasteurization.

Each basket was given the number of the test to which it was assigned (52 to 56) and a letter to designate the treatment (table 1).

TABLE 1.—*Description of treatments given sweetpotatoes*

Treatment designation	Pasteurization period	Humidity during pasteurization	Length of curing period	Treatment designation	Pasteurization period	Humidity during pasteurization	Length of curing period
	Days		*Days*		*Days*		*Days*
A (check)	0		10	Pasteurization at 110° F.—Continued			
Pasteurization at 110° F.:				G	3	Low	10
B	1	High	10	H	1	High	0
C	1	Low	10	I	2	do	0
D	2	High	10	J	2	do	3
E	2	Low	10	K	2	Low	3
F	3	High	10				

After the curing period all lots were moved into the storage room, where they remained until they were examined.

RESULTS

SWEETPOTATOES FROM A FIELD FREE OF BLACK ROT

The variety Orange Little Stem from a field free of black rot was used in test 52. The results given in table 2 show the general effects of the treatments on loss of weight through transpiration and respiration during pasteurizing, curing, and subsequent storage for 10 weeks; also, the percentage of sweetpotatoes (on basis of original weight) that remained healthy or affected with soft rot,[3] end rot caused by *Fusarium*, and other rots. No black rot developed in this test.

TABLE 2.—*Effect of pasteurizing and curing treatments on Orange Little Stem sweetpotatoes not infected with black rot and stored for 10 weeks*

[Average of 3 replicates]

Test and treatment [1]	Original weight per half-bushel lot	Weight loss during—			Roots sound [2]	Weight loss from—		
		Pasteurizing and curing	Storage	Pasteurizing, curing, and storage		Soft rot (*Mucor* type)	End rot	Other rots
	Pounds	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
52A	27.4	4.4	8.6	13.0	85.2	0.7	0.9	0.0
52B (101)	23.5	5.8	8.4	14.2	79.6	2.1	2.0	.3
52C (room)	27.4	6.2	7.7	13.9	80.1	3.5	1.6	.5
52D (100)	23.1	8.1	7.1	15.2	78.8	3.2	.7	1.9
52E (room)	27.2	6.6	7.9	14.5	75.6	5.7	2.9	.7
52F (100)	23.5	9.1	7.5	16.6	70.9	9.5	1.7	.3
52G (room)	26.1	9.0	7.3	16.3	71.4	8.8	.8	1.0
52H (101)	23.6	1.1	18.8	19.9	41.0	9.2	27.8	1.3
52I (101)	22.7	3.5	16.6	20.1	24.1	25.7	28.6	.9
52J (100)	23.5	5.8	9.0	14.8	68.8	3.3	10.3	2.2
52K (room)	27.3	4.4	8.0	12.4	73.8	7.2	3.7	2.0

[1] See table 1 for details of treatments; 100 and 101 designate high-humidity pasteurization chambers.

[2] Difference required for significance at 5-percent level, 9.4 percent. The statistical analysis was made without converting percentages to angles, because the percentage figures ranged between 20 and 90.

[3] Soft rot in this paper refers to a soft, watery rot similar to that developing at low temperatures and probably caused by an organism of the *Mucor* type. A study of the rot organism is being made.

In this test at 110° F. the relative humidity in the chambers (high) fluctuated around 80 percent and in the room (low) around 60 percent (fig. 1). Under these conditions weight loss and rot development were sufficiently similar for comparable treatments. Loss in weight increased during pasteurization as well as during curing, but subsequently it tended to become equalized during storage for all cured lots. Although the check (treatment A) showed the greatest percentage of healthy stock when examined January 15, this lot was not significantly better than those pasteurized 1 or 2 days. Lack of significance for treatment E, however, is questionable. The 3-day pasteurization caused a material reduction in keeping quality. Pasteurization at 110° for 1 or 2 days without curing was not effective; other lots with only a 3-day curing after 2 days of pasteurizing kept nearly as well as those similarly pasteurized but cured a full 10-day period.

The capacity of these sweetpotato roots to produce sprouts, or draws, together with that of those in test 56 (p. 189), is shown in table 3. The roots in test 52 were bedded January 16 in moist sand in shallow boxes and placed under artificial light in the room originally used for curing (85° F. and 80 percent relative humidity). The number of sprouts that developed was determined February 2. After this period of 17 days under good conditions most of the sprouts above the sand were several inches long.

Table 3 shows that 1 day of pasteurizing followed by 10 days of curing did not lessen the production of sprouts in comparison with curing only; but pasteurizing for 2 or 3 days caused a reduction in sprout production whether the roots were cured 3 or 10 days. Whether this situation would hold true at normal bedding time is not known.

To test the effect of the pasteurizing treatment on culinary quality, five representative sweetpotatoes from each of the treatments were baked and tasted. A bitter or off flavor was found in roots given treatments D, E, F, G, I, and K; those given treatments A, B, and C were judged satisfactory and those given treatments B and C less so than those given A.

TABLE 3.—*Sprout production by pasteurized and nonpasteurized roots of Orange Little Stem and Maryland Golden sweetpotatoes*

Variety, test, and treatment [1]	Weight of 9 roots bedded	Sprouts produced—			Roots dormant	Roots rotted
		Above sand	Below sand	Total		
Orange Little Stem:	*Pounds*	*Number*	*Number*	*Number*	*Number*	*Number*
52A	2.3	99	118	217	0	0
52B	2.0	74	138	212	0	1
52C	2.1	114	156	270	0	0
52E	2.0	43	75	118	0	3
52G	2.0	54	93	147	0	4
52K	2.0	27	77	104	0	1
Maryland Golden:						
56A	2.5	63	33	96	0	0
56B	2.6	71	37	108	0	0
56C	2.4	38	130	168	0	0
56D	2.5	41	24	65	0	3
56E	2.7	49	40	89	[2] 1	0
56G	2.9	57	56	113	0	1

[1] See table 1 for details of treatments.
[2] Partly rotted on each end.

COMMERCIALLY WASHED SWEETPOTATOES THAT CONTAINED SOME BLACK ROT AT DIGGING

Because of their similarity in material, methods, and results, tests 53 and 55 are considered together. Test 53 was started October 24 and test 55 October 1, and the roots were examined January 7 and 8, respectively. Maryland Golden sweetpotatoes that had been washed commercially were used in both tests. The roots used in test 53 were placed under test conditions the day they were dug; those in test 55 were obtained from a packing shed where they had been for an undetermined length of time after digging. Both lots contained some black rot when delivered to the shed. All visibly infected potatoes were removed after the roots were washed. In all check lots (A) some black rot developed during the storage period, but not a single black rot lesion could be found in any of the pasteurized lots (table 4). This indicates that sweetpotatoes infected with black rot or carrying black rot inoculum can be effectively pasteurized and that this disease can be arrested by subjecting the roots to 110° F. for 1 day. The effect of the pasteurizing treatment on the development of other rots is not clear.

TABLE 4.—*Kind and amount of decay developing in pasteurized and nonpasteurized washed Maryland Golden sweetpotatoes that were cured*

Test and treatment [1]	Total roots tested	Roots sound	Roots affected with—					Mummies
			Black rot	Soft rot (*Mucor* type)	End rot	Surface rot [2]	Other rot	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
53A	68	32	18	14	3	0	0	1
53B (102)	24	24	0	0	0	0	0	0
53C (room)	36	24	0	9	3	0	0	0
53D (102)	41	25	0	1	15	0	0	0
53E (room)	44	11	0	1	31	0	1	0
55A [3]	311	28	23	174	38	1	0	47
55B (102) [3]	137	56	0	17	34	22	0	8
55C (room) [3]	134	32	0	41	25	3	0	33

[1] See table 1 for details of treatments; 102 designates a high-humidity pasteurization chamber.
[2] Surface rot could easily have been classed as desiccated skinned areas or bruises on healthy potatoes.
[3] 55A represents 6 replicates; 55B and 55C, 3 replicates each.

SWEETPOTATOES WITH BLACK ROT LESIONS PRESENT

In test 54 an attempt was made to kill the black rot organism after it was well established. On Maryland Golden sweetpotatoes that had black rot lesions at the time of digging the margins of the lesions were carefully outlined with an indelible pencil before the roots were treated. About 1 peck of such sweetpotatoes was used for each treatment. This test was begun October 24 and the roots were examined January 7, when they were divided into three classes: (1) Those on which the lesions had enlarged beyond the indelible line; (2) those on which the lesions had not enlarged beyond that line; and (3) those so badly rotted by other organisms that it was impossible to tell what had happened to the black rot lesions (table 5 and fig. 2) On only one root from the pasteurized lots did rot develop beyond the indelible line, and it was doubtful whether black rot was the cause of this

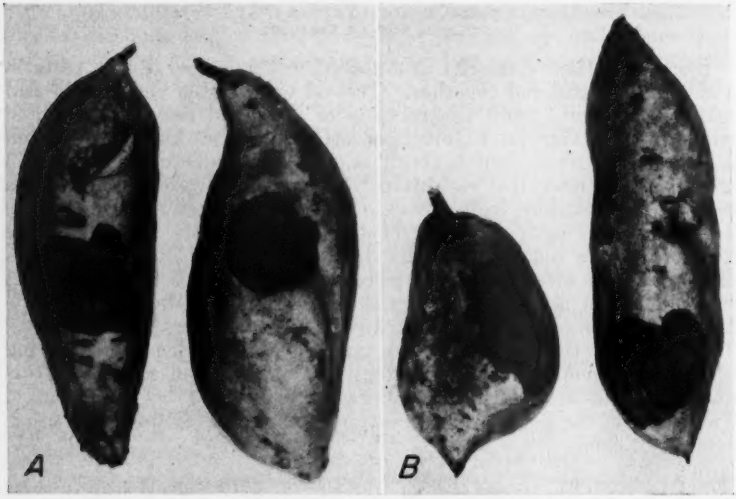

FIGURE 2.—*A*, Black rot lesions on sweetpotatoes pasteurized 1 day, which did not enlarge; *B*, lesions on nonpasteurized sweetpotatoes, which enlarged during storage.

extension, because isolations from it did not yield the black rot fungus. When isolations were made from the enlarged lesions on the check lots, however, several cultures of the black rot fungus were obtained; but none was obtained from the lots pasteurized for 1 day. It was noted also that the lesions on pasteurized roots remained shallow like those on freshly dug potatoes, whereas some of the enlarged lesions on the check lots had progressed into the tissue of the potato for as much as half an inch. Some of the other lesions did not develop beyond the stage they had reached at the time of treatment. These were shallow and appeared to be inactive or dead, like those on the pasteurized lots, indicating that some of the black rot lesions found at digging do not remain active.

TABLE 5.—*Enlargement of black rot lesions on pasteurized and nonpasteurized roots of Maryland Golden sweetpotatoes during 10 weeks in storage*

Test and treatment [1]	Potatoes tested	Margins of lesions extended	Margins of lesions not extended	Lesions obscured by other rots
	Number	*Number*	*Number*	*Number*
54A–1	16	[2] 7	3	6
54A–2	17	[2] 5	6	6
54B (102)	16	0	[3] 13	3
54C (room)	16	[3] 1	[2] 8	7
54D (102)	18	0	15	3
54E (room)	15	0	8	7
54F (102)	15	0	5	10
54G (room)	15	0	10	5

[1] See table 1 for details of treatments; 102 designates a high-humidity pasteurization chamber.
[2] Isolations yielded black rot fungus.
[3] Isolations did not yield black rot fungus.

UNWASHED SWEETPOTATOES THAT CONTAINED SOME BLACK ROT AT DIGGING

In test 56, started October 29 and examined January 8, Maryland Golden sweetpotatoes from a field where black rot was in evidence were put through the same series of pasteurizing and curing. Careful records were made of weight losses and types of rots that developed. All roots having black rot lesions at time of digging were sorted out and thrown away. Unlike the sweetpotatoes in tests 53 and 55, these were not washed. Although these roots came from a field where only a few roots were infected with black rot, in the three check baskets an average of 8 percent by weight of the original lot developed the disease (table 6). The percentages of sound roots except after the 3-day pasteurization, while not greatly increased in the pasteurized lots, indicate that had the black rot taken a greater toll the pasteurized lots would have had considerably more sound roots; or, if the surface rots had been considered as desiccated bruises and classed as sound, all the pasteurized lots, except those given treatment G, would have had considerably larger percentages of healthy roots. Results for treatment F are omitted from the table because the 3 days at high humidity and 110° F. induced to a marked degree a type of decay occasionally noticed in similarly treated roots in the other tests—that is, a shallow, pitlike rot probably caused by a species of *Rhizopus*. In treatment 56F this did not stop and dry up as in the other lots pasteurized 3 days but continued until nearly every infected potato was destroyed.

TABLE 6.—*Effect of pasteurizing and curing treatment on Maryland Golden sweetpotatoes infected with black rot and stored for 10 weeks*

[Average of 3 replicates]

Test and treatment [1]	Original weight per half-bushel basket	Weight loss during—			Roots sound	Weight loss from—			
		Pasteurizing and curing	Storage	Pasteurizing, curing, and storage		End rot	Surface rot [2]	Black rot	Other rots
	Pounds	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
56A	22.3	3.5	2.7	6.2	76.5	5.4	2.9	8.0	0.0
56B (102)	21.7	5.4	3.7	9.1	77.7	4.1	7.6	.0	.8
56C (room)	20.0	5.5	3.7	9.2	84.2	2.3	2.9	.0	1.0
56D (102)	20.8	7.0	2.8	9.8	85.4	1.9	2.6	.0	.0
56E (room)	21.7	7.4	3.1	10.5	76.9	3.4	5.8	.0	3.4
56G (room)	22.2	8.3	4.5	12.8	74.2	1.5	6.5	.0	5.1

[1] See table 1 for details of treatments; 56F was discarded November 13, 1947, because of excessive rotting; 102 designates a high-humidity pasteurization chamber.

[2] Surface rot could easily have been classed in most cases as desiccated skinned areas or bruises on healthy potatoes.

In considering the effect of these pasteurizing treatments upon the development of other rots it was noted that prolonged treatment of potatoes was detrimental, and that the type of rot that developed seemingly depended on factors other than the pasteurization treatment itself.

Some roots from each of these treatments were bedded January 12 and were examined for sprouts February 2. The results indicate again that pasteurization for 1 day did not hurt the sprouting capacity

of the potatoes (table 3). However, these data are not so clear-cut as those for Orange Little Stem (table 3). Irregularity of sprouting was also noted in other tests with Maryland Golden.

SUMMARY AND CONCLUSIONS

In sweetpotatoes freshly dug from fields where black rot occurred subjection to 110° F. for 1 day or more and then curing at 85° and 80 percent humidity prevented the development of black rot in storage and killed the causal fungus on lesions present when dug. In roots treated for more than 1 day at this temperature an off flavor was found in baked lots, sprout production was inhibited, and frequently certain other types of decay were prevalent. If treated for only 1 day at 110° the sweetpotatoes were not materially damaged in any way.

A FURTHER TOXICOLOGICAL COMPARISON OF DERRIS AND LONCHOCARPUS [1]

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In a recent article [2] a report was made of a toxicological comparison of some authentic samples of roots of *Derris* and *Lonchocarpus* varieties. In the work therein described several types of *Derris* and *Lonchocarpus* were used, each of which was represented by one or two samples of roots grown in the same location. The report showed derris root to be generally superior to lonchocarpus root even when the rotenone content was equal. The present report deals with a similar comparison of the same species with some of the types represented by samples grown under different conditions. Additional chemical analyses were made in an attempt to trace the toxicity due to compounds other than rotenone.

The varieties of *Derris elliptica* (Roxb.) Benth. used were: Sarawak Creeping, St. Croix, and Changi No. 3. The Sarawak Creeping variety was represented by samples of roots grown in Puerto Rico and of roots grown in Guatemala. The Changi No. 3 was represented by samples of the Rio Piedras clone and the Mayagüez-Goodyear (MG) clones. The lonchocarpus root used was of *Lonchocarpus utilis* A. C. Smith and *L. chrysophyllus* Kleinh. Of *L. utilis*, one sample was from plants grown in Puerto Rico and one from plants grown in South America. This gave a set of 8 root samples, each of which represented 10 or more plants.

The toxicological tests were made on houseflies (*Musca domestica* L.) and Mexican bean beetles (*Epilachna varvivestis* Muls. The samples for the tests with houseflies were prepared as in the previous study,[3] and the dilutions used were such that 1 ml. of the test solutions contained 0.09 mg. of rotenone. The tests were carried out by the turntable method on adult houseflies reared by standard procedure. Eight tests were made on each sample with about 150 flies to each test. For reference, tests were also made with solutions of 5 graded concentrations of pure rotenone. The toxicity of the rotenone standards was plotted against concentration on log-probability paper and a straight line fitted. The rotenone equivalents of the root samples were then obtained by reference to this graph. The results are given in table 1.

[1] Received for publication June 25, 1948.

[2] JONES, M. A., GERSDORFF, W. A., and MCGOVRAN, E. R. A TOXICOLOGICAL COMPARISON OF DERRIS AND LONCHOCARPUS. Jour. Econ. Ent. 39: 281–283. 1946.

[3] See footnote 2, page 196.

TABLE 1.—*Comparative toxicity of some derris and longchocarpus samples to houseflies*

Sample and where obtained	Concentration [1]	Mortality of flies in 3 days [2]	Rotenone equivalent of test solution	Rotenone equivalent of root	
				Total	Rotenoids
D. elliptica:	*Mg./ml.*	*Mean percent*	*Mg./ml.*	*Percent*	*Percent*
Sarawak Creeping, Puerto Rico	1.69	46	0.222±0.015	13.14±0.89	7.74
Sarawak Creeping, Guatemala	1.71	33	.146± .011	8.59± .64	3.29
Changi No. 3, MG	1.25	35	.153± .012	12.24± .92	4.94
Changi No. 3, Rio Piedras	2.44	35	.153± .012	6.24± .49	2.54
St. Croix	4.99	48	.236± .016	4.73± .32	2.93
L. utilis:					
Puerto Rico	1.64	32	.140± .011	8.54± .67	3.04
South America	1.91	37	.171± .013	9.00± .68	4.30
L. chrysophyllus	1.55	23	.106± .009	6.84± .58	1.14
Rotenone	.1	23	.100± .009		
	.2	40	.200± .013		
	.4	68	.400± .034		
	.8	81	.800± .070		
	1.6	91	1.60 ± .23		

[1] Concentration of oven-dry, powdered root, the concentration of rotenone in each test solution was 0.09 mg. per milliliter.

[2] Standard error of mean mortalities is 2.2 percent. Differences between mean mortalities greater than 6 percent are significant at odds of 19:1.

For the tests on the Mexican bean beetle, the spray tower-petri dish technique was used. This method was designed to duplicate some of the practical conditions that would be encountered in control work in the field. Excised bean leaves with petioles in vials of water were treated uniformly on both sides by a falling mist spray consisting of a suspension of the powdered sample in water containing acacia. The amount of sample in the spray was adjusted for each sample so that the rotenone concentration was 0.0125 ± 0.0002 percent. To prepare the control samples, an acetone solution of rotenone was mixed thoroughly with fuller's earth and the acetone evaporated. The powder was then passed through a 200-mesh sieve and suspended in water containing the wetting agent. As soon after the spraying as the water had evaporated from the surface the leaves were placed in 6-inch petri dishes and infested with 10 third instar larvae of the Mexican bean beetle. The larvae were confined in the dish by a 16-mesh screen cover. After 3 days the treated leaves were removed and an unsprayed leaf placed in each dish. This was done to permit larvae that were only slightly poisoned by the spray to feed on fresh foliage and recover. Thus those repelled by the spray deposit would not starve and so give a false idea of the value of the insecticide. It was felt that this practice resembles practical conditions under which, about 3 days after spray treatment, there is usually untreated leaf area present on bean plants, due to new growth, opening of flowers, or removal of spray deposit by weathering. However, the technique differed from practical conditions in that the spray was not applied directly to the insect as it would be to a great extent in spraying a bean field. Also under practical conditions larvae only slightly poisoned may fall off the plant and be killed by predators, trampling, or desiccation. Therefore, under the conditions of the tests the larvae had a better opportunity to recover from slight poisoning.

The spray residue may have acted as a contact insecticide on the insects that crawled on it. It also acted as a stomach poison when the leaf was fed upon by the larvae. The dosage received by a larva was also probably affected by the repellent action of some constituents of the spray. Final mortality counts were made on the sixth day. The entire series was run in duplicate daily for 10 days to give a total of 20 tests for each of the 8 samples and 2 controls. In order to determine how much of the leaf was fed upon, the area eaten was estimated by placing a grid on the leaf and counting the number of square centimeters missing. Where only portions of a square centimeter were eaten, the area was estimated. The results of the tests are given in table 2.

TABLE 2.—*Comparative toxicity of some derris and lonchocarpus samples to Mexican bean beetle larvae*

Sample and where obtained	Concentration in spray	Amount eaten [1]	Mortality in 6 days [2]	Calculated mortality per cm.² consumed	
				Rotenone [3]	Total root [4]
D. elliptica:	*Gm./100 ml.*	*Cm.² test*	*Percent*	*Percent*	*Percent*
Sarawak Creeping, Puerto Rico	0.23	1.6	49	31	33
Sarawak Creeping, Guatemala	.24	3.2	42	13	14
Changi No. 3, MG	.17	1.9	37	19	28
Changi No. 3, Rio Piedras	.34	2.0	37	18	13
St. Croix	.69	2.1	44	21	8
L. utilis:					
Puerto Rico	.23	4.3	27	6	7
South America	.27	4.1	39	10	9
L. chrysophyllus	.22	6.3	32	5	6
Rotenone preparation [5]	.25	5.0	40	8	8
Rotenone preparation [6]	.25	3.8	38	----	10

[1] 5.0 cm.² is a significant difference.
[2] 10 percent is a significant difference.
[3] Equal dosages of rotenone.
[4] Equal dosages of root (0.25 gm. per 100 milliliter).
[5] Standard 5 percent rotenone in fuller's earth.
[6] Standard 20 percent rotenone in fuller's earth.

Chemical examination of the samples consisted of rotenone analyses by the method of the Association of Official Agricultural Chemists.[4] The procedure outlined by Goodhue and Haller [5] was used to determine the alkali-soluble fraction, fats and waxes, and the neutral resin. The content of rotenone plus rotenoids was determined by a

[4] ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS . . . Ed. 5, 757 pp., illus. Washington D. C. 1940. (See pp. 64–66.)

[5] GOODHUE, L. D., and HALLER, H. L. THE NON-CRYSTALLINE CONSTITUENTS OF TEPHROSIA VIRGINIANA ROOTS. Amer. Chem Soc. Jour. 62: 2520–2522. 1940.

colorimetric procedure [6] and deguelin by the method of Goodhue and Haller.[7]

The tests with houseflies allow the samples to be ranked as follows with regard to toxic value: (1) Derris Sarawak Creeping from Puerto Rico and derris Changi No. 3, MG clone, (2) the two *Lonchocarpus utilis* samples and the derris Sarawak Creeping from Guatemala, (3) *L. chrysophyllus* and derris Changi No. 3, Rio Piedras clone, and (4) derris St. Croix.

The mortality of larvae on the foliage, treated or untreated, varied considerably. In trials with untreated foliage, 8 tests early in the period of testing gave 2 percent average mortality, 6 tests near the end of the period gave 40 percent, and 16 tests in the final week gave 72 percent. The data for rotenone in fuller's earth show clearly that the toxicity was not proportional to the rotenone content of the spray; a 5-percent rotenone preparation gave the same kill as a preparation containing 20 percent. Likewise, in tests not shown in table 2, 1.25 percent rotenone in fuller's earth gave 62 percent kill, while a preparation in which pure rotenone was used gave only 70 percent kill. From these results it is apparent that little increase in effectiveness resulted from increasing the concentration of rotenone in the spray. The larvae apparently compensated for the increased rotenone by reducing their feeding and contact. That the amount of feeding decreased as the concentration of rotenone was increased was shown by the following tests which were made in pairs: With 1.25 percent rotenone, 6.0 cm.^{2} was eaten, and with 98 percent, 1.2 cm.^{2}; with 2.5 percent, 14.1 cm.^{2}, and with 10 percent, 9.1 cm.^{2}; with 5 percent, 5 cm.^{2}, and with 20 percent, 3.8 cm.^{2}. Therefore, since it was not possible to arrive at rotenone equivalents as was done with the data for houseflies, the comparison was made on the basis of mortality in 6 days with equal rotenone dosages and the amount of feeding was taken into account.

The data indicate that the derris samples caused higher mortality than the lonchocarpus and permitted less feeding when equal dosages of rotenone were used. On the basis of mortality per unit area consumed the Puerto Rican Sarawak Creeping and the Changi No. 3 MG roots appear to be the most effective and the St. Croix derris and the lonchocarpus samples the least.

The rotenone equivalents calculated from the toxicological tests on houseflies are shown in table 3, together with the results of chemical examination.

Since it was not possible to calculate rotenone equivalents from the data with the bean beetle, only the data from the tests with houseflies are included for comparison with those obtained by chemical examination. Such comparison brings out several points. It shows (1) that deguelin was not the only toxicant besides rotenone and (2) that the

[6] JONES, M. A. THE APPLICATION OF A MODIFIED RED-COLOR TEST FOR ROTENONE AND RELATED COMPOUNDS TO DERRIS AND LONCHOCARPUS. Assoc. Off. Agr. Chem. Jour. 28: 352–359, illus. 1945.

[7] GOODHUE, L. D., and HALLER, H. L. A METHOD FOR DETERMINING DEGUELIN IN DERRIS AND CUBE. Indus. and Engin. Chem., Analyt. Ed. 11: 640–642. 1939.

alkali-soluble fractions and the fats and waxes had no appreciable toxicity. The compounds that contribute the extra toxicity apparently occur in the neutral resin fraction. Toxicity over that due to rotenone was roughly proportional to the neutral resin content. The percentages of neutral resin indicate that it contained varying amounts of toxicant.

TABLE 3.—*Chemical analysis and rotenone equivalent of some derris and lonchocarpus samples as determined by tests on houseflies*

Sample		Chemical analyses (dry basis)									
Type	Where grown	Plants represented	Total extractives	Rotenone plus rotenoids	Rotenone	Alkali-soluble fraction	Fats and waxes	Neutral resins	Deguelin	Toxicarol and tephrosin	Rotenone equivalent of roots
		Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
D. elliptica:											
Sarawak Creeping.	Puerto Rico	162	16.4	12.7	5.4	0.32	1.41	10.49	0.16	0.12	13.14
Do	Guatemala	80	12.6	9.9	5.3	.33	.89	7.08	.....	1.05	8.59
Changi No. 3, MG.	Puerto Rico	191	16.6	13.5	7.3	.48	2.45	7.31	.22	1.29	12.24
Changi No. 3, Rio Piedras.	do	129	7.8	6.3	3.7	.34	1.43	3.86	.....	.75	6.24
St. Croix	do	854	5.9	4.6	1.8	.37	1.13	3.81	.13	.78	4.73
L. utilis	do	10	8.3	6.4	5.5	.22	1.97	2.43	.....	.05	8.54
Do	South America.	32	12.4	8.8	4.7	.67	1.73	4.02	2.64	3.27	9.00
L. chrysophyllus	Puerto Rico	10	7.7	6.2	5.7	.37	1.05	2.06	.....	.32	6.84

Inasmuch as such roots are valued for their insecticidal action and accurate biological tests are expensive on a large scale, it may be of value to rank the samples according to toxicity and compare this order with that obtained by use of the chemical analytical criteria. Such a comparison is shown in table 4.

From this table it can be seen that ranking according to the content of rotenone plus neutral resin or according to total chloroform extractives agrees most closely with the order obtained by tests with houseflies. Neither of these criteria gives numerical values which agree even closely with the numerical values for rotenone equivalent; for numerical agreement the colorimetric determination for rotenone plus rotenoids gives the best agreement but it fails to bring out small differences.

With regard to the two types represented by samples grown in different localities, it is clear that the toxicity per unit of rotenone content was considerably different in the same variety grown in two localities; this indicates that growing conditions, as well as variety, affect the toxicity of the nonrotenone constituents of the roots.

TABLE 4.—*Rank of samples according to several criteria* [1]

Sample and where obtained	Rotenone equivalent	Rank based on—				
		Toxicity to houseflies	Total chloroform extractives	Rotenone plus rotenoids	Rotenone	Rotenone plus neutral resin
D. elliptica: Sarawak Creeping, Puerto Rico	*Percent* 13. 14	1 or 2	1 or 2	2	2, 3, 4, or 5	1
Changi No. 3 MG	12. 24	1 or 2	1 or 2	1	1	2
L. utilis: South America	9. 00	3, 4, or 5	3 or 4	4	6	4
D. elliptica: Sarawak Creeping, Guatemala	8. 59	3, 4, or 5	3 or 4	3	2, 3, 4, or 5	3
L. utilis: Puerto Rico	8. 54	4 or 5	5	5, 6, or 7	2, 3, 4, or 5	5, 6, or 7
L. chrysophyllus	6. 84	6 or 7	6 or 7	5, 6, or 7	2, 3, 4, or 5	5, 6, or 7
D. elliptica: Changi No. 3, Rio Piedras	6. 24	6 or 7	6 or 7	5, 6, or 7	7	5, 6, or 7
St. Croix	4. 73	8	8	8	8	8

[1] Note that the rank is not based on a uniform scale but rather on relative order among the 8 samples, 1=highest, 8=lowest.

SUMMARY

Nine chemical characteristics and the toxicity to two species of insects were determined for eight samples of derris and lonchocarpus root. When the samples were ranked on the basis of toxicity, rotenone plus natural resins and total chloroform extractives were found to be most nearly in accord with their toxicity to houseflies. The rotenone plus rotenoids ranking was fairly similar to the insecticidal ranking; rotenone content as well as the other chemical characteristics determined showed considerable divergence from the toxicological value.

In general, derris had a higher insecticidal value than lonchocarpus when comparisons were based on equal rotenone content. The derris grown in Puerto Rico was much more toxic than that grown in Guatemala, but there was little difference in the lonchocarpus samples from Puerto Rico and South America.

The housefly was more satisfactory than the larvae of the Mexican bean beetle as a test insect for evaluation of the relative toxicity of these samples. However, despite the dissimilarity in the manner in which the insecticides were applied, the rankings according to toxicity to the two species agreed when the differences between samples were relatively great.

COMPARISON OF CHEMICAL VALUES WITH THE TOXICOLOGICAL ROTENONE EQUIVALENT OF DERRIS AND LONCHOCARPUS ROOTS [1]

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INTRODUCTION

During the recent war rotenone was an important item on the list of scarce and strategic materials of the United States. The main source of supply was cut off when the Japanese invaded the principal derris-producing areas of the world. As a result, large quantities of *Lonchocarpus* or cube roots were imported from Peru and other South American countries, where this rotenone-bearing species grows extensively. Table 1 shows the relative percentages of derris and

TABLE 1.—*United States imports of derris and lonchocarpus roots, from 1941 to 1946* [1]

Species	1941	1942	1943	1944	1945	1946 [2]
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Lonchocarpus root	48.7	73.0	100	100	99.4	99.8
Derris root	51.3	27.0			.6	.2

[1] From a report by Lawrence Witt (*10*), Office of Foreign Agricultural Relations, U. S. Department of Agriculture.
[2] Data for 1946 from Claud L. Horn of the same office.

lonchocarpus root imported into the United States between 1941 and 1946.

Assuming that the inventories of most importers are small from year to year, it is evident from these data that practically the entire amount of rotenone used in the United States during the past 4 years was obtained from the roots of lonchocarpus. The results of a field experiment performed by Dr. G. E. R. Hervey of the New York State Agricultural Experiment Station [2] indicate that insecticidal preparations made from derris may be more toxic than those made from lonchocarpus at the same rotenone level. In Hervey's experiment samples of powdered derris and lonchocarpus root each containing 5 percent rotenone were diluted with talc to make dusts containing 1.0, 0.5, and 0.25 percent rotenone. These dusts were applied to cabbage having a light infestation of the imported cabbageworm (*Ascia rapae* (L.)) and cabbage looper (*Autographa brassicae* (Riley)).

[1] Received for publication June 25, 1948.
[2] Unpublished data.

The dusts were applied three times at an average rate of 25 pounds per acre with an interval of about 2 weeks between applications. The results, which are shown in table 2, are based on the quantity of foliage

TABLE 2.—*Comparative control of the imported cabbageworm and cabbage looper with rotenone dust from derris and lonchocarpus roots*[1]

Source	Rotenone	Average injury index	Control
	Percent	*Percent*	*Percent*
Derris	1.0	17.1	70.2
Lonchocarpus	1.0	21.5	62.5
Derris	.5	22.1	61.5
Lonchocarpus	.5	25.0	56.4
Derris	.25	29.1	49.3
Lonchocarpus	.25	28.8	49.8
Check		57.4	
Least difference required for significance (19:1)		3.3	

[1] Unpublished data from G. E. R. Hervey, New York State Agricultural Experiment Station.

consumed and were taken when insect activity had ceased. These data show that the insect control obtained with 1.0 percent rotenone dust made from derris root was significantly better than that obained with dust of the same rotenone content made from lonchocarpus. The insect control obtained with 0.5 percent rotenone dust made from derris was better, although not significantly so, than that made from lonchocarpus. The control with 0.25 percent rotenone dust made from derris and lonchocarpus was about the same. In commercial practice these insecticidal plants are evaluated and sold mainly on the basis of their rotenone content, but this criterion apparently does not indicate their toxicological value.

In 1944 Pepper and Filmer (9)[3] reported results of insecticidal tests in which an insecticidal plant of low rotenone content, *Derris malaccensis* Prain, was compared with other species of *Derris* containing a higher rotenone content. Their data showed a higher toxicological value for *D. malaccensis* than for the other species. More recently Jones et al. (*5*) have presented data which show that "* * * the colorimetric determination of rotenone plus rotenoids was the best chemical estimate of the toxicity of the samples, although it varied considerably from the biological evaluation."

A criterion other than rotenone percentage is needed to establish the true toxicological value of these insecticidal plants. Such a criterion, to be used commercially or when large numbers of samples are involved, must of necessity be simple, rapid, and reasonably accurate. Pagán and Loustalot (*8*) suggested the use of transmittance values of acetone extracts of derris roots measured in a spectrophotometer at 360 mμ. These measurements correlated fairly closely with the rotenone equivalent as determined biologically. However, this method has certain limitations due to the fact that special apparatus and technique are needed. Likewise, the red-color value, suggested by Jones (*4*), also has limitations because a colorimeter or other suitable instrument is needed, the conditions required for the test are rather exacting, and the results are not always in close agreement with the biological assays.

[3] Italic numbers in parentheses refer to Literature Cited, p. 204.

Total extractives as a measure of toxicity have not been used extensively because these values are generally higher and thus do not closely approximate the true insecticidal value of the roots. Also, in comparing some of these chemical analyses with results of tests on insects, derris and lonchocarpus samples frequently were included in the same experiments. In this paper data from derris and lonchocarpus samples are presented which show a comparison between some chemical analyses such as total extractives, red-color values, and rotenone and the rotenone equivalent (determined biologically). In these comparisons the data from derris and lonchocarpus samples are treated separately.

MATERIALS AND METHODS

Thirteen rotenone samples were available for comparison. Eight of these were from roots of *Derris elliptica* and 5 were *Lonchocarpus* (3 of *L. utilis* and 2 of *L. chrysophyllus*) (*5*, *6*). All samples were assayed biologically on houseflies (*Musca domestica* L.) by W. A. Gersdorff and E. R. McGovran of the Bureau of Entomology and Plant Quarantine.[4] These 13 samples were used because the essential data were at hand; data on more samples were desired but no other biologically assayed samples were available. Rotenone analyses were made according to the procedure of the Association of Official Agricultural Chemists (*2*, *pp. 64–66*), and total chloroform extractives were determined from an aliquot of the chloroform extract as obtained in the rotenone determination. The procedure described by Jones (*4*) was used to determine the red-color values. The regression equation and the correlation coefficient between the biologically determined rotenone equivalent and each of the afore-mentioned chemical values were calculated.

RESULTS

Table 3 gives the correlation coefficients and regression equations calculated between the toxicological rotenone equivalent of derris samples and the three chemical values of rotenone, red-color value, and total chloroform extractives. Although all three correlation coefficients were highly significant, the values for total chloroform extractives and red-color value were much higher than that for rotenone, indicating that total chloroform extractives and red-color value are better criteria. The t values obtained indicated highly significant regression coefficients in all cases, but again the value for total chloroform extractives (10.19) and for red-color value (10.98) were both considerably higher than the value for rotenone (4.36). The standard error of estimate calculated for the three analytical values show that the error in estimating toxicity from rotenone is about twice that from total chloroform extractives and red-color value.

[4] Merriam A. Jones, formerly chemist at this station, also participated in the chemical analyses herein reported.

TABLE 3.—*Correlation coefficients, standard error of estimates, and regression equations between toxicological rotenone equivalent and analytical values of some derris and lonchocarpus samples*

Constituent	Correlation coefficient [1]		Standard error of estimate		Regression equations [2]	
	Derris roots	Lonchocarpus roots	Derris roots	Lonchocarpus roots	Derris roots	Lonchocarpus roots
Total chloroform extractives	0.972	0.435	1.20	1.01	$T=0.932+0.868E$	$T=6.090+0.165E$
Red-color value	.972	.300	1.22	1.01	$T=1.0620-0.709C$	$T=6.490+0.1610C$
Rotenone	.877	−.832	2.47	.64	$T=1.471+1.730R$	$T=18,160-1.920R$

[1] For the derris samples correlation coefficients were highly significant (1-percent level) in all cases, but for the lonchocarpus samples they were not significant.
[2] The regression coefficients of the equation were significant at the 1-percent level for the derris samples, but not significant for the lonchocarpus samples. In these equations T=toxicological rotenone equivalent, C=red-color value, E=total chloroform extractives, and R=rotenone.

The regression lines between the rotenone equivalent of the derris samples and the rotenone content, red-color value, and total chloroform extractives, were plotted (fig. 1) from values obtained from the equations presented in table 4. It is evident from these graphs that the best agreement with rotenone equivalent is obtained with total chloroform extractives, and the next best with red-color value.

TABLE 4.—*Comparison of biological rotenone equivalent of 8 derris samples with that calculated from total chloroform extractives, red-color value, and rotenone content*

Sample No.	Variety	Rotenone equivalent [1]	Total chloroform extractives		Red-color value		Rotenone	
			Actual	Calculated [2]	Actual	Calculated [2]	Actual	Calculated [2]
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
D-1	Changi No. 3, MG clone	18.7	22.3	18.4	18.2	18.6	10.0	18.8
D-2	Sarawak Creeping	15.7	16.8	13.7	13.5	13.6	5.7	11.3
D-3	do	13.1	16.4	13.3	12.7	12.8	5.4	10.8
D-4	Changi No. 3, MG clones	12.2	16.6	13.5	13.5	13.6	7.3	14.1
D-5	Changi No. 3, P. R. clones	9.4	12.4	9.8	10.2	10.1	5.3	10.6
D-6	Sarawak Creeping	8.5	12.6	10.0	9.9	9.8	5.3	10.6
D-7	Changi No. 3, P. R. clone	6.3	7.8	5.8	6.3	6.0	3.7	7.9
D-8	St. Croix	4.7	5.9	4.2	4.6	4.2	1.8	4.6

[1] Determined biologically on houseflies.
[2] These values are rotenone equivalents calculated from the respective regression equations.

It is interesting to note that if the regression line between rotenone equivalent and rotenone (fig. 1, *A*) is projected until it intersects the abscissa axis a hypothetical root is found with some toxicity and no actual rotenone content. Actually, such a root has been reported by Pepper and Filmer (*9*), who found that a simple of *Derris malaccensis* root had only a trace of rotenone but relatively high toxicological value.

This is additional evidence that at least with derris roots the rotenone content is not a dependable criterion of the insecticidal value of the roots. When the regression line obtained between red-color value and rotenone equivalent was projected (fig. 1, *B*), it intersected the ordinate axis, indicating that a hypothetical case may be found in which there is some red-color value but no toxicity. No instance of this

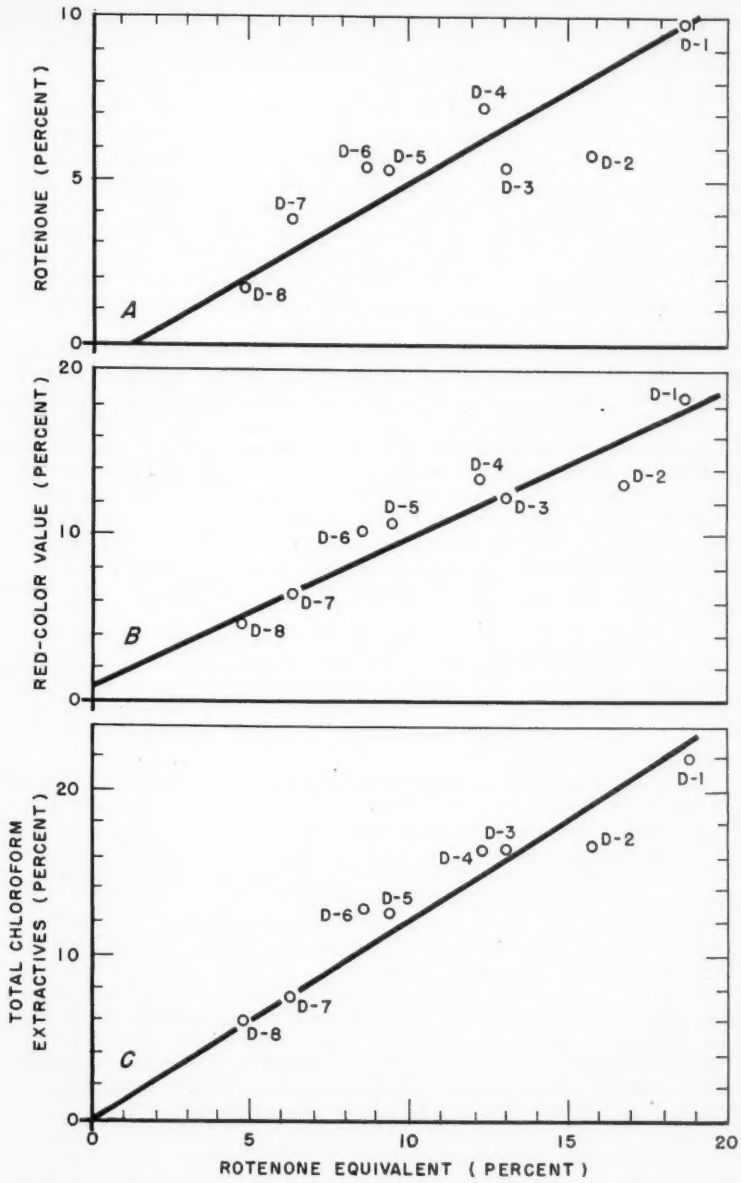

FIGURE 1.—Plot of regression lines between rotenone equivalent and rotonone content (*A*), red-color value (*B*), and total chloroform extractives (*C*) of eight derris samples.

sort has been reported for derris, but Acree et al. (*1*) found that *Amorpha fruticosa* L. gave a red color but did not contain rotenone. Likewise, Norton and Hansberry (*7*) isolated a nontoxic rotenoidlike compound from yam beans (*Pachyrhizus erosus* (L.) Urban) which gave a positive reaction to the Goodhue red-color test. Apparently, there may be substances in some roots that give the red-color test but have no toxicological value. When the regression line obtained with total chloroform extractives was projected (fig. 1, *C*), the line intersected the origin. This fact furnishes further evidence that total chloroform extractives are a better criterion of toxicity than red-color value or rotenone. This finding is in agreement with that of Ebeling et al. (*3*), who showed that the complete extractives were a more effective insecticide than equal concentrations of either the rotenone or the rotenone-free extractives used by themselves.

Table 4 shows a comparison between the toxicological rotenone equivalent of some derris samples and the values obtained from the regression equation of each of the chemical criteria. It is evident from this table that rotenone is the least accurate criterion of toxicity. The values for rotenone varied as much as 65 percent from the rotenone equivalent as determined biologically. Total chloroform extractives and red-color value were more closely in agreement with the toxicological value. The former varied as much as 17 percent and the latter up to 13 percent from the rotenone equivalent. If these deviations are compared with the error of the biological method, which in some cases was as high as 9 percent, they cannot be considered excessive.

There was no statistically significant correlation or regression between the rotenone equivalent and any of the constituents studied in lonchocarpus roots. The correlation and regression between rotenone and rotenone equivalent was almost negatively significant with a value of $r=-0.832$. For significance at the 5-percent level the correlation coefficient should be 0.878.

Although there was no significant regression between toxicological rotenone equivalent and any of the chemical values, regression equations were calculated and regression lines plotted. It can be seen from figure 2 that total chloroform extractives and red-color value do not fit the line closely. However, rotenone is in good agreement with the regression line. This indicates that rotenone, although it correlates negatively, is the best indication for toxicity in lonchocarpus roots. But in view of the small number of samples used this conclusion can only be regarded as tentative.

In table 5 are presented for comparison the toxicological rotenone equivalent of the lonchocarpus samples and the values calculated from the regression equation of each of the chemical criteria. The least deviation from rotenone equivalent is found in the values calculated from rotenone (11 percent) followed by those from total chloroform extractives (13 percent) and red-color value (14 percent).

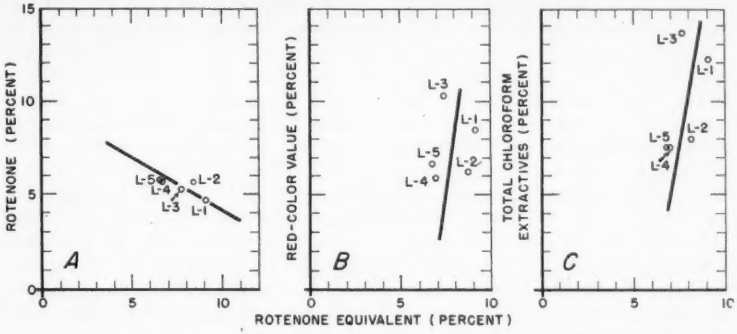

FIGURE 2.—Plot of regression lines between rotenone equivalent and rotenone content (*A*), red-color value (*B*), and total chloroform extractives (*C*) of five lonchocarpus samples.

TABLE 5.—*Comparison of biological rotenone equivalent of 5 lonchocarpus samples with that calculated from rotenone content, red-color value, and total chloroform extractives*

Sample No.	Variety	Rotenone equivalent [1]	Rotenone		Red-color value		Total chloroform extractives	
			Actual	Calculated [2]	Actual	Calculated [2]	Actual	Calculated [2]
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
L-1	*L. utilis*	9.0	4.7	9.1	8.8	7.9	12.4	8.1
L-2	do	8.5	5.5	7.6	6.4	7.5	8.3	7.5
L-3	do	7.7	5.4	7.8	10.6	8.2	14.0	8.4
L-4	*L. chrysophyllus*	6.8	5.7	7.2	6.2	7.5	7.7	7.4
L-5	do	6.7	5.8	7.0	7.0	7.6	7.8	7.4

[1] Determined biologically on houseflies.
[2] These values are rotenone equivalents calculated from the respective regression equations.

DISCUSSION AND CONCLUSIONS

Insofar as the results presented here are applicable to field conditions in general, it is evident that rotenone content is not an accurate indication of the toxicological value of derris roots. It is a well-established fact that rotenone is an efficient and versatile insecticide, but it is also apparent that it is only one of several constituents in derris roots which are capable of killing insects. The best criterion of toxicity would appear to be one which would measure not only rotenone but the other toxic constituents as well. The red-color value seems to correlate more closely with rotenone equivalent than rotenone content alone because it includes such rotenoid type compounds as deguelin. However, since there may be other toxic substances in the root which do not give the red-color test, this criterion in some instances may not indicate the total toxicological value of the roots. The red-color value has the added disadvantage, as pointed out previously, of requiring special equipment and also rather exacting conditions. On the other hand, total chloroform extractives are very

simple to determine and require a minimum of time and equipment. In addition to this, the total chloroform extractives appear to be the most accurate criterion of toxicity of derris roots. This apparently is because all, or almost all, of the insecticidal constituents of derris roots are soluble in chloroform and, therefore, are readily extracted by this solvent. Since waxes, resin, and other plant material may dissolve in chloroform it is not surprising that the value for total chloroform extractives as such is usually somewhat higher than that of the rotenone equivalent as determined biologically. However, from the correlation coefficient obtained it is apparent that the ratio of the nontoxic constituent of the derris root to the toxic constituents is fairly constant and thus the total chlorform extractives multiplied by the appropriate factor give a good indication of the insecticidal value of the roots. The factor calculated from the eight available derris samples was 0.78.

In the case of lonchocarpus, neither the total chloroform extractives nor the red-color value appears to be a very good criterion of toxicity, whereas rotenone content, although it correlates negatively with toxicological value, seems to be more indicative. However, since the number of samples available for comparison is relatively small, this may not always be the case.

Although both *Lonchocarpus* and *Derris* contain rotenone, they are two distinct genera and apparently have different toxicological effects on insects. The data presented in this paper strongly suggest that they should be evaluated and treated separately.

SUMMARY

The data presented furnish additional evidence that rotenone content is not an accurate indication of the toxicological value of derris roots.

The red-color and the transmittance values give a good indication of the toxicity of derris root, but they have the disadvantage of requiring a spectrophotometer and special technique, and thus are not suitable for rapid assays.

The percentage of total chloroform extractives appears to be the simplest and most accurate criterion for estimating the toxicity of derris roots. In the eight derris samples tested the toxicity (rotenone equivalent) was 0.78 of the value for total chloroform extractives.

In the case of lonchocarpus root, neither the total chloroform extractives nor the red-color or transmittance values appear to be very good criteria of toxicity. The rotenone content, although negatively correlated with toxicological value, seems to be more indicative.

The results of these laboratory tests and of those from practical field trials strongly suggest that derris and lonchocarpus roots, although they both contain rotenone, should be evaluated separately.

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